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Autonomic Nervous System Reactivity in Early Childhood: Developmental Patterns and Sociodemographic Predictors

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Autonomic Nervous System Responsivity in Early Childhood: Developmental Patterns and Sociodemographic Predictors

by

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DISSERTATION

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Autonomic Nervous System Reactivity in Early Childhood:

Developmental Patterns and Sociodemographic Predictors

Michelle Stephens

Abstract

Cardiac autonomic nervous system (ANS) measurements, respiratory sinus arrhythmia (RSA) and preejection period (PEP), are valid and reliable indicators of children's sensitivity to stressors in their environment; however, there are few studies of RSA and PEP measures in children younger than three years of age and no known studies of children at 18-months of age.

This was a cohort study of racially- and ethnically-diverse, low-income children studied from birth through five years of age. At 18-months (n = 134) and 36-months (n = 102) of age, children completed a developmentally challenging protocol that simultaneously assessed their RSA and PEP under resting and challenge conditions. Reactivity was calculated as the difference between challenge and resting measures. Four ANS profiles were created by dichotomizing reactivity, mean challenge minus rest, as positive or negative reactivity.

This study revealed novel information about the distribution, stability, and continuity of RSA and PEP rest, challenge, and reactivity measures. There was a significantly different distribution of children among the ANS profiles from 18- to 36-months of age; although, there was some stability for the reciprocal PNS activation/SNS not activated profile. The relations between selected sociodemographic characteristics (biological sex, race, ethnicity, and federal poverty level (FPL)) and ANS profiles showed that their prevalence differed at 18- and 36-months of age. Logistic regression models revealed significant relationships between FPL and the coactivation and coinhibition profiles at 18-months and a borderline significant relationship between being Hispanic and the reciprocal SNS activation/PNS withdrawal profile at 36-months.

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Chapter One: Introduction

Stress is an inevitable part of life affecting every human being beginning from conception. One sensitive biomarker of stress is the autonomic nervous system (ANS), which innervates and regulates the body's visceral organs. Cardiac measurements of the ANS are highly responsive, valid, reliable, and sensitive to changes that occur in an individual's environment (Alkon et al., 2003; Berntson, Quigley, & Lozano, 2007). Furthermore, ANS measures elucidate the individual's unique expression of their stress response.

The autonomic nervous system (ANS) is the part of the body's nervous system mainly responsible for controlling and regulating organ and tissue activity, usually under both conscious and unconscious control. The ANS consists of two branches, the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS). The PNS is the "rest and restorative" system that facilitates a restorative state while sleeping or relaxing. The SNS is the "fight or flight" system that is aroused to respond to emergencies or threatening situations (Berntson et al., 2007). Specific cardiac measures, respiratory sinus arrhythmia (RSA) and preejection period (PEP), can assess both the withdrawal of the PNS and activation of the SNS, respectively.

Respiratory sinus arrhythmia (RSA), an index of the parasympathetic influence on the ANS, accounts for the change in heart rate (HR) due to the frequency of respirations (Berntson et al., 2007; Porges 1995, 2007). Decreases in RSA indicate that vagal nerve stimulation is withdrawing and subsequently there is a decrease in HR corresponding to a faster respiratory rate, indicating PNS withdrawal. PNS withdrawal or vagal suppression, as measured by RSA, is related to positive social engagement (Porges et al., 1996) and has been found to predict emotion regulation and internalizing and externalizing problems later in life (Zisner & Beauchaine, 2016).

Preejection period (PEP), a measure of the time interval from the onset of ventricular depolarization to the onset of left ventricular ejection, reflects the influence of the SNS on cardiac activity (Berntson et al., 2007). Decreases in PEP or shortened time intervals indicate that the ventricular myocardium is being activated by the SNS. Increased PEP reactivity has been associated with attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and conduct disorder (CD) in preschool-age children and delinquency, aggression, or obsessive behaviors in adolescents (Beauchaine et al., 2001; Beauchaine et al., 2013; Beauchaine, Hong, & Marsh, 2008; Brenner & Beauchaine, 2011; Crowell et al., 2006;).

RSA and PEP can be measured under resting and challenge conditions and combined to describe reactivity, the difference between a challenge and a resting condition (Matthews et al., 2002). Reactivity has different meaning for PNS and SNS measures, with four key categories defined as follows (Alkon et al., 2017):

- *High RSA reactivity* (i.e., negative RSA difference score) indicates PNS withdrawal (withdrawal of the vagus nerve) during the challenges compared to the resting state.
- *High PEP reactivity* (i.e., negative PEP difference score; shortening of the PEP during the challenges compared to rest) indicates more SNS activation (increased “fight or flight” impulse) during the challenges compared to the resting state.
- *Low RSA reactivity* (i.e., positive RSA difference score) indicates more PNS activation (i.e., more vagal input, or promotion of a relaxed state) during the challenge compared to the resting state.
- *Low PEP reactivity* (i.e., positive PEP difference score; lengthening of the PEP during the challenges compared to rest) indicates SNS not activated during the challenge compared to the resting state.

Historically, the autonomic nervous system (ANS) response was conceptualized as a single continuum, with sympathetic activation ("fight or flight" impulse) on one end and parasympathetic activation (relaxation state) on the other. Then the concept of autonomic space was introduced, and it characterized autonomic responses into a two-dimensional model, with SNS activity and PNS activity as separate axes within this space (Berntson et al., 1991). Working within this two-axis model, Berntson et al. (1991) researchers have proposed different modes of autonomic control, reciprocal, nonreciprocal (coactivation or coinhibition), or uncoupled activity, that fall into six categories: coactivation, coinhibition, uncoupled parasympathetic changes, uncoupled sympathetic changes, reciprocal parasympathetic activation, and reciprocal sympathetic activation. Because unilateral (i.e. uncoupled) parasympathetic and sympathetic activation profiles are not common in children, a four-profile categorization has been demonstrated most commonly in children (Salomon et al., 2000; Alkon et al., 2003). These four profiles are conceptualized as follows:

- *Coactivation*: the SNS and PNS are both activated in challenge (versus rest) conditions, indicated by a positive RSA reactivity score and a negative PEP reactivity score. An increased "fight or flight" impulse occurs simultaneously with promotion of a relaxed state.
- *Coinhibition:* the SNS and PNS are both inhibited in challenge conditions, indicated by a negative RSA reactivity score and a positive PEP reactivity score. A decreased "fight or flight" impulse occurs along with withdrawal from a relaxed state.
- *Reciprocal parasympathetic (PNS) activation/sympathetic (SNS) not activated:* the SNS is inhibited and the PNS is activated in challenge conditions, indicated by a positive RSA

reactivity score and a positive PEP reactivity score. Promotion of a relaxed state occurs with decreased "fight or flight" impulse.

- *Reciprocal sympathetic (SNS) activation/ parasympathetic (PNS) withdrawal (aka classic reactivity):* the SNS is activated and the PNS is inhibited in challenge conditions, indicated by a negative RSA reactivity score and a negative PEP reactivity score. Increased "fight or flight" impulse occurs along with withdrawal from a relaxed state.

The understanding of PNS and SNS responsivity, through the cardiac measures of RSA and PEP resting, challenge, and reactivity, is the foundation of this research. From which, ANS reactivity profiles are formed and these profiles can then be looked at in relationship to early childhood characteristics to better understand the influences that the environment may have on ANS responsivity.

Literature Review

A review of the literature reveals normal distributions within ANS study samples (Alkon et al, 2003; Alkon et al., 2011; Bush et al., 2017); however, these studies used different data collection methods (e.g., protocols), hardware (e.g., acquisition equipment), and analysis software compared to each other.

The reliability or stability of ANS responsivity within an individual has been reported in a few studies (Alkon et al., 2003; Gatzke-Kopp & Nam, 2018; Hinnant et al., 2011; Matthews et al., 2002). Overall, these studies showed consistent, moderate stability of RSA and PEP resting but a lack of stability of RSA and PEP reactivity. Overall, these studies show similar RSA and PEP values between similar age groups.

Previous studies found that RSA resting measures show a gradual increase during the first six years of life (Alkon et al., 2006; Alkon et al., 2011; Bar-Haim, Marshall, & Fox, 2000;

Bornstein & Suess, 2000; Calkins & Keane, 2004). PEP reactivity increased from three to eight years of age (Alkon et al., 2003) and from eight to 17 years of age (Matthews et al., 2002). Overall, children's RSA and PEP reactivity levels appeared to change with age, indicative of developmental changes or different challenge conditions. It is not known if RSA and PEP reactivity responsivity becomes more consistent at older ages.

Since the early 2000s, a number of researchers have examined autonomic reactivity in samples of children and adolescents and have assigned reactivity profiles defined by both PNS and SNS activity. Though exact protocols have differed across studies, all the studies reviewed have assessed children's cardiac activity during both age-appropriate challenge and resting conditions. The studies reviewed in this dissertation have employed two main systems to define profiles. Several have followed the four-profile version of the Berntson et al. (1991) categories as previously defined. Several recent studies assigned ANS profiles consistent with a newer Adaptive Calibration Model (ACM), (Ellis et al., 2017; Kolacz et al., 2016; Del Giudice et al., 2012). The ACM proposed four patterns (sensitive, buffered, vigilant, and unemotional); the sensitive pattern displays high PNS and moderate SNS responsivity, the buffered pattern has more PNS activity compared to SNS activity, the vigilant pattern has high SNS and low PNS responsivity, and the unemotional pattern has low PNS and SNS responsivity. These ACM patterns can be tangentially matched with the Berntson et al. (1991) ANS profiles to assist with a holistic interpretation of the studies thus far that have looked at the combined effect of PNS and SNS activity. The general aims of this body of literature to date have been to characterize ANS reactivity patterns at different ages, to explore whether adverse early experiences (e.g. family conflict) are associated with children's reactivity patterns, and to describe behavioral characteristics of children in different profiles.

According to the Gene x Environment Interaction model, the physical, chemical, and built environmental factors can affect adult health by becoming biologically embedded during sensitive developmental periods (Hertzman, 2013b), such as in early childhood, and/or accumulate damage over time (Johnson, 2005; Shonkoff, 2010). Hertzman's theory of biological embedding explains that if children grow up in healthy environments, they would be less likely to have early disease onset and the society would have smaller disparities in health status and income (Hertzman & Boyce, 2010). There is evidence that children who are male (Solomon, Matthews, & Allen, 2000), African American (Wagner et al., 2016), or living in poverty (Alkon et al., 2011; Ellis, Essex, & Boyce, 2005) have increased PEP reactivity. Therefore, the relationship between the environment and young children's ANS responsivity is an important intersection to study in the field of psychophysiological research. Environmental influences, such as demographic characteristics, are often underreported in psychophysiological research (Gatzke-Kopp, 2016). Phenotypes such as biological sex, race, ethnicity, and socioeconomic status, may have a role in understanding the process by which the autonomic nervous system responds and develops over time.

Gaps and Significance

Measuring ANS activity (resting) and reactivity (challenge minus resting) can be related to both normal and pathological functioning of learning, behavioral, and health disorders (Zisner & Beauchaine, 2016). While these previous studies provided a solid foundation for understanding ANS measures in childhood, there is still little known about the ANS of children from infancy to three years of age. Although ANS measures provide important markers of stress physiology, few studies have examined them among young children, including their distribution, stability, and continuity.

To date, there are studies on ANS reactivity that do not acquire measurements from a single organ (i.e., the heart) (Beauchaine, 2009). About 33% of the reviewed studies used SCL (derived from the skin) or sAA (derived from an accessory organ) as their proximal measurement of SNS activity. Not collecting ANS reactivity measurements from a single organ can decrease the validity and reliability of the study. Repeated cardiac measurements of ANS reactivity in children younger than three years of age are rarely collected as Alkon et al. 2011 study has been the only one to date. The evaluation of ANS reactivity profiles has rarely been done. Furthermore, RSA and PEP has not been previously studied in 18-month-old children.

Overall, understanding how stress affects a young child needs to be addressed because of the vulnerability of the young child (Weiss, 2015). Young children are vulnerable because they do not have the ability to control their environment, which is built around them with little to no opportunity for them to change it on their own. Today's young children will be tomorrow's teachers, medical providers, and civic leaders. The health of our country depends on the health of today's young children. Additionally, children who grow up unhealthy will disproportionately need support from social systems. The investment in early childhood outcomes is important and cost-effective (Braveman & Barclay, 2009; Heckman, 2006).

Study Aims

This dissertation entitled, "Autonomic Nervous System Reactivity in Early Childhood: Developmental Patterns and Sociodemographic Predictors", has the following study aims: 1) to describe the distribution, stability, and continuity of RSA and PEP during resting, challenge, and reactivity states, in a sample of predominantly minority children from low-income families assessed longitudinally at 18- and 36-months of age, 2) to describe the proportion of children at 18 and 36 months of age in the four ANS reactivity profiles (i.e., a) coactivation, b) coinhibition,

c) reciprocal PNS activation/SNS not activated, d) reciprocal SNS activation/PNS withdrawal); and, assess the stability of the four ANS reactivity profiles from 18 months to 36 months of age; and, 3) to identify the relationship between selected sociodemographic characteristics (children's biological sex, race, ethnicity, and federal poverty level (FPL)) and children's ANS reactivity profiles (coactivation, coinhibition, reciprocal PNS activation/SNS not activated, and reciprocal SNS activation/PNS withdrawal) at 18- and 36-months of age.

Study Design and Sample

This dissertation is a secondary analysis of the birth cohort study, the Stress, Eating, and Early Development (SEED) study, which was a continuation of the Maternal Adiposity, Metabolism, And Stress (MAMAS) study. The MAMAS study was a non-randomized control trial designed to examine the effects of a mindfulness-based stress reduction and healthy lifestyle intervention to reduce excessive gestational weight gain among pregnant women (Epel et al., 2019); the SEED study followed the children of MAMAS mothers from birth through age five, investigating the impact of prenatal and early childhood stress and eating habits on the children's behavioral, physiologic, and anthropometric development (Bush et al., 2017).

Children included in these analyses were 19.0 months old (SD = 1.3) on average, at the time of their 18-month-old visit (n = 134), and were 38.7 months (SD = 3.4) at the time of their 36-month-old visit (n = 102); 53% of participants at both visit points were girls. Based on the parent's classification of their child's race, the participants included in the 18-month-old visit analysis were 32% White, 14% were Black, and 54% Other (e.g. Asian, Native American, mixed race); 42% were classified as Hispanic. Among children included in 36-month-old visit analyses, 36% were White, 12% were Black, and 52% were Other, with 33% of the total further classified as Hispanic. The mean household federal poverty level was less than 200% at the 18-month and 36-month-old visits, with a range of 15% – 794% .

Manuscripts

The first manuscript presented in this dissertation titled, “Distribution, Stability, and Continuity of Autonomic Nervous System Responsivity at 18- and 36-Months of Age” summarizes the sample distributions, stability, and continuity of children’s RSA and PEP measures during resting, challenge, and reactivity for children at 18- and 36-months.

The second manuscript titled, “Autonomic Nervous System Reactivity in Early Childhood: Developmental Patterns from 18- to 36-Months of Age” identifies four ANS reactivity profiles that combine RSA and PEP reactivity based on activation or withdrawal of the parasympathetic (PNS) or sympathetic nervous system (SNS) respectively (coactivation, coinhibition, reciprocal PNS activation/SNS not activated, and reciprocal SNS activation/PNS withdrawal) and assesses the stability of the profiles from 18- to 36-months of age.

The third and final manuscript, “The Sociodemographic Context of Autonomic Nervous System Reactivity in Early Childhood” identifies the relationship between selected sociodemographic characteristics (children’s biological sex, race, ethnicity, and federal poverty level (FPL)) and children’s ANS reactivity profiles (coactivation, coinhibition, reciprocal PNS activation/SNS not activated, and reciprocal SNS activation/PNS withdrawal) at 18- and 36-months of age.

The dissertation concludes with a summarization of the findings and a discussion of what the findings mean for the field of early childhood stress physiology.

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Chapter Two: First Manuscript

Distribution, Stability, and Continuity of Autonomic Nervous System Responsivity at 18- and 36-Months of Age

Abstract

Background: Cardiac autonomic nervous system (ANS) measurements, respiratory sinus arrhythmia (RSA) and preejection period (PEP), are valid and reliable indicators of children's sensitivity to stressors in their environment; however, there are few studies of RSA and PEP measures in children younger than three years of age.

Objectives: This study's aim was to summarize the sample distributions, stability, and continuity of children's RSA and PEP measures during resting, challenge, and reactivity for children at 18- and 36-months.

Methods: This was a cohort study of racially- and ethnically-diverse, low-income children studied from birth through five years of age. At 18-months (n = 134) and 36-months (n = 102) of age, children completed a developmentally challenging protocol that simultaneously assessed their RSA and PEP under resting and challenge conditions. Reactivity was calculated as the difference between challenge and resting measures.

Results: At 18- and 36-months of age, 53% of participants were girls, 32-36% were White, 12-14% were Black, 52-54% were Other (Asian, Native American, and mixed race), 33-42% classified as Hispanic, and the mean household was < 200% of the federal poverty level. RSA and PEP resting, challenge, and reactivity measures were normally distributed at each age. RSA resting, RSA challenge, PEP resting, and PEP challenge from 18- to 36- months of age were positively and moderately correlated, but RSA and PEP reactivity were positively weakly correlated. There were statistically significant mean changes for RSA and PEP resting, challenge, and reactivity from 18- to 36-months of age.

Discussion: Individual children showed moderate stability in RSA and PEP during rest and challenge, but not for reactivity scores, from 18- to 36- months. This diverse sample showed a lack of continuity in the mean RSA and PEP resting, challenge, and reactivity measures from 18- to 36-months of age supporting ANS changes across development. These findings support the need for further study of RSA and PEP responsivity in young children from diverse backgrounds to understand the factors that may contribute to neurobiological development during early childhood.

*Keywords***:** stress, early childhood, autonomic nervous system, respiratory sinus arrhythmia, preejection period, development

Introduction

Children who experience prolonged or excessive stress during early childhood are at risk of both short- and long-term health problems, including learning, behavior, and physical health problems (Alkon et al., 2011; Beauchaine et al., 2013; Bauer et al., 2002; Boyce et al., 1995; Calkins & Keane, 2004; Loman & Gunnar, 2010; Pearson et al., 2005; Shonkoff et al., 2014; Treadwell et al., 2011). The theory of biological embedding explains that experiences can change children's brain architecture, immunologic responses, and genomic function affecting both current and future health (Hertzman, 1999). Hertzman (2013a) also suggested that adversity experienced during certain timepoints in childhood, known as sensitive developmental periods, may be particularly damaging and long-lasting.

Cardiac measures of the autonomic nervous system (ANS) have been shown to be valid and reliable indicators of children's sensitivity to everyday stressors in their environment (Bernston et al., 2007; Alkon et al., 2003). Furthermore, ANS measures can elucidate a child's unique physiologic expression of their stress response, which indicates their individual differences of psychobiologic responsivity. As such, cardiac measurements of the ANS are important indicators of individual differences early in life as physiologic stress responses, as well as measures of biological embedding.

The ANS, which consists of the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS), is one of the most responsive physiologic systems to stress (Bernston et al., 2007). The ANS is the part of the body's nervous system mainly responsible for controlling and regulating organ activity, usually under both conscious and unconscious control. The PNS is the "rest and restorative" system that facilitates a restorative state while sleeping or relaxing. The SNS is the "fight or flight" system that is aroused to respond to emergencies or threatening situations (Berntson et al., 2007). Specific cardiac measures, respiratory sinus arrhythmia (RSA) and preejection period (PEP), can assess both the activation and withdrawal of the PNS and SNS, respectively.

Respiratory sinus arrhythmia (RSA), an index of the parasympathetic influence on the ANS, accounts for the change in heart rate (HR) due to the frequency of respirations (Berntson et al., 2007; Porges 1995, 2007). Decreases in RSA indicate that vagal nerve stimulation is withdrawing. This involves a subsequent decrease in HR as influenced by a faster respiratory rate, a process defined as PNS withdrawal. PNS withdrawal or suppression, as measured by RSA, is related to positive social engagement (Porges et al., 1996) and has been found to predict emotion regulation and internalizing and externalzing disorders later in life (Zisner & Beauchaine, 2016).

Preejection period (PEP), a measure of the time interval from the onset of ventricular depolarization to the onset of left ventricular ejection, reflects the influence of the SNS on cardiac activity (Berntson et al., 2007). Decreases in PEP or shortened time intervals indicate that the ventricular myocardium is being activated by the SNS. Increased PEP reactivity has been associated with attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and conduct disorder (CD) in preschool-age children and delinquecy, aggression, or obsessive behaviors in adolescents (Beauchaine et al., 2013; Crowell et al., 2006; Beauchaine et al., 2001, Beauchaine, Hong, & Marsh, 2008; Brenner & Beauchaine, 2011).

RSA and PEP can be measured under resting and challenge conditions and combined to describe reactivity, the difference between a challenge and a resting condition (Matthews et al., 2002). Measuring ANS activity (resting) and reactivity (challenge minus resting) can be related to both normal and pathological functioning of learning, behavioral, and health disorders (Zisner

& Beauchaine, 2016). Although ANS measures provide important markers of stress physiology, few studies have examined them among young children, including their distribution, stability, and continuity. Stability and continuity provide complementary insight of developmental changes over time. Stability offers information about individual change where continuity offers information about group mean change over time.

Distribution of ANS Measures in Early Childhood

Distributions of ANS measures show all of the possible values for a measure and how they are spread across the sample population (Shott, 1990). Studies consistently find that RSA and PEP are normally distributed (Alkon et al, 2003; Alkon et al., 2011; Bush et al., 2017). Normal distributions are evidence of a pattern of individual differences with different samples of young children showing a range from high to low responses. Although the majority of ANS studies show normal distributions within their samples, these studies used different data collection methods (e.g., protocols), hardware (e.g., acquisition equipment), and analysis software compared to each other.

A few studies of RSA and PEP resting, challenge, and reactivity measures in early childhood reported similar means but they used different data collection procedures and/or equipment and scoring software. In a cross-sectional study of 3-5-year-olds (N = 51) in child care centers, a 15-minute standardized protocol with resting and challenging conditions was administered while continuous measures of RSA and PEP responsivity were measured (Alkon et al., 2003). This sample was primarily Caucasian boys, families with middle to high incomes, and mothers with at least a college degree. Their mean RSA resting (M = 5.8, SD = 1.2) and PEP resting (M = 77.5, SD = 6.6) were similar to the control group in a cross-sectional study of children with ADHD and ODD (N = 18, 7 girls) with RSA resting (M = 6.7, SD = 1.4) and PEP resting (*M* = 71.5, *SD* = 14.9). Age-matched controls (*N* = 20, 9 girls) with a mean age of 4.5 years old were administered a 30-minute protocol with a five minute baseline, with the remainder of the time spent playing a "Perfection" game used to elicit an ANS response to challenge and reward (Crowell et al., 2006). Notably, this study also reported on the control group's RSA challenge (*M* = 6.1, *SD* = 1.2) which was similar to Alkon et al. (2003) 3-5 year old study group's RSA challenge (*M* = 5.6, *SD* = 1.1).

A cohort study of low-income, primarily Latino infant's 6- and 12-month-old RSA resting and reactivity were assessed using a 7-minute ANS protocol and the Minnesota Impedance Cardiograph HIC-2000 for data acquisition and ANS Suite software (Mindware Technologies, LTD) to collect and analyze the data (Alkon et al., 2011). Jewell, Suk, & Leucken (2017) reported on RSA resting at 6- and 12-months among a sample of low-income Mexican American infants who completed a 7-minute baseline protocol using electrocardiogram (ECG) equipment from Forest Medical, LLC hardware and CardioBatch software to score RSA. Alkon et al. (2011) study reported at 6-months: RSA resting (*M* = 3.3, *SD* = 0.8) and RSA reactivity (*M* = 0.01, *SD* = 0.5); and at 12-months: RSA resting (*M* = 3.7, *SD* = 0.9) and RSA reactivity (*M* = 0.1, *SD* = 0.6) while Jewell, Suk, & Leucken (2017) reported similar results for RSA resting at 6 months (*M* = 3.1, *SD* = 0.9) and RSA resting at 12 months (*M* = 3.7, *SD* = 1.0). A cohort study of primarily Latino, low-income 3- to 5-year-old children (Alkon et al., 2014) had similar RSA and PEP resting means and standard deviations to the previous Alkon et al. (2003) study with primarily Caucasian, middle to high income 3- to 5-year-old children. In both studies, the children completed the same ANS protocol with four challenging tasks and a rest period. However, the data were acquired on different equipment (Biopac versus Minnesota Impedance) but analyzed with Mindware software. Overall, these studies show similar RSA and PEP values between similar age groups; however, these studies utilized different types of equipment, software, and protocols.

Stability of ANS Measures in Early Childhood

Stability is defined as the "consistency in relative ranks of individuals in a group with respect to a function or process through time" (Bornstein & Suess, 2000, p. 54) or their between-individual variability. Instability would then refer to the change of their relative order in a group over time (Bornstein, Putick, & Esposito, 2017). The reliability or stability of ANS responsivity within an individual has been reported in a few studies (Hinnant et al., 2011; Alkon et al., 2006; Matthews et al., 2002).

In early and middle childhood, RSA indices during resting and challenge (without accounting for baseline levels) does appear to be stable at the between-individual level while RSA reactivity appears not to be stable, especially during infancy. Hinnant, Elmore-Staton, and El-Sheikh (2011) studied developmental stability of the PNS and SNS in 8- ($N = 251$) to 10- ($N = 185$) year-olds (162 European American and 89 African American) over three waves (when the children were 8-, 9- and 10-years-old). The children were given an acclimating period of six minutes, to help adjust to their new environment, followed by a three-minute resting period. There were two challenges presented (argument and star tracing task) and a 12-minute recovery period between each challenge. This study only reported on the stability over three timepoints for RSA and PEP resting. There was positive, moderate stability from each consecutive timepoint for RSA and PEP (Hinnant et al., 2011). A study of primarily, low-income Mexican-American children from infancy to five years of age ($n = 297$) were given a 7- (6- and 12-month-olds) or 15-minute (42- and 60-month-olds) developmentally appropriate challenges protocol with continuous RSA and PEP measures (Alkon et al., 2006). Moderate stability was found over time for RSA resting (intraclass correlation (*ICC*) = 0.3) and RSA challenge (*ICC* = 0.3) and PEP resting (*ICC* = 0.4) and PEP challenge (*ICC* = 0.5) but not RSA or PEP reactivity (Alkon et al., 2006). The Solomon, Matthews, & Allen (2000) study recruited 8-10-year-old children (*N* = 201), 48% Black and 52% White and 50% male. They participated in a protocol with three challenges: the reaction time task, a mirror tracing task, and the Social Competence Interview (Salomon et al., 2000). RSA and PEP resting and challenge showed positive, moderate stability and no stability of RSA and PEP reactivity (Solomon et al., 2000). Overall, these studies showed consistent, moderate stability of RSA and PEP resting but a lack of stability of RSA and PEP reactivity.

Continuity of ANS Measures in Early Childhood

Continuity refers to the “consistency in the absolute level of a function or process in a group through time” (Bornstein & Suess, 2000, p. 54) or the mean level change of the construct over time within a sample (Hinnant et al., 2018). Previous studies found that RSA resting measures show a gradual increase during the first six years of life (Alkon et al., 2006; Alkon et al., 2011; Bar-Haim, Marshall, & Fox, 2000; Bornstein & Suess, 2000; Calkins & Keane, 2004). PEP reactivity increased from three to eight years of age (Alkon et al., 2003) and from eight to 17 years of age (Matthews et al., 2002). In a cohort study of Mexican American children from six months through five years, Alkon et al. (2011) reported that, on average, RSA reactivity increased as children got older, suggesting that the stress response became more robust as children developed (i.e., children exhibited PNS withdrawal in response to challenges versus rest). These findings on the sample’s continuity in ANS responsivity were supported by Conradt et al. (2014) with a longitudinal sample of children three to six years of age but differed in a study of three to five-year-olds where there was no significant change in RSA reactivity (Perry et al., 2013). Overall, children's RSA and PEP reactivity levels appeared to change with age, indicative of developmental changes or different challenge conditions. It is not known if RSA and PEP reactivity responsivity becomes more consistent at older ages.

Children's neurobiological development has been shown to demonstrate plasticity, sensitivity to their environment, and rapid growth particularly in the first three years of life. This study was the first that we are aware of to report on both RSA and PEP measures at 18-months of age. There were several studies that measured RSA and PEP during infancy using the Still Face Paradigm (SFP) (Jones-Mason et al., 2018) and during the preschool-ages but not toddlers. As we cannot assume a linear path of ANS development from 6- months to 3-years of age (Koss & Gunnar, 2018), this gap in knowledge needed to be addressed. While these previous studies provided a solid foundation for understanding ANS measures in childhood, there is still little known about the ANS of children from infancy to three years of age. The aim of this study was to describe the distribution, stability, and continuity of RSA and PEP during resting, challenge, and reactivity states, in a sample of predominantly minority children from low-income families assessed longitudinally at 18- and 36-months of age.

Methods

This cohort study is part of a larger birth cohort study, the Stress, Eating, and Early Development (SEED) study, which was a continuation of the Maternal Adiposity, Metabolism, And Stress (MAMAS) study. The MAMAS study was a non-randomized control trial designed to examine the effects of a mindfulness-based stress reduction and healthy lifestyle intervention to reduce excessive gestational weight gain among pregnant women; the SEED study followed the children of MAMAS mothers from birth through age five, investigating the impact of prenatal and early childhood stress and eating habits on the children's behavioral, physiologic, and anthropometric development (Bush et al., 2017). This paper describes and analyzes the ANS data collected at the 18-month and 36-month visits.

Participants in the MAMAS study were pregnant women recruited between August 2011 and June 2013 when they were between their 16th and 22nd week of pregnancy. Women were eligible to participate if they were of low to middle income and overweight or above. Recruitment took place at hospital-based clinics; community health centers; Supplemental Nutrition Program for Women, Infants and Children (WIC) offices; organizations providing services to pregnant women; and through online advertisements (e.g., Craigslist). In total, 220 women enrolled in the MAMAS study, of whom 215 remained involved in the study at delivery of a liveborn child. All of the 215 mothers were invited to enroll themselves and their newborns in the follow-up SEED study at delivery. In total, 180 mother-child dyads (84%) enrolled in SEED, of whom 134 children (74% of the enrolled delivery sample) and 102 children (57% of the delivery sample) participated fully at 18-month and 36-month study visits, respectively.

Data Collection

For both the 18- and 36-month visits, a trained research assistant met with the mother and child in the family's home and the dyad were together during the study visit. During these visits, the research assistant administered multiple questionnaires with the mother, took anthropometric measurements of the child, and conducted a standardized, age-specific Developmental Challenges Protocol (DCP) with the child while simultaneously monitoring their ANS activity (RSA and PEP).

The research assistant placed four spot electrodes on the child's neck and trunk to collect impedance and respiratory measures and three spot electrodes were placed on the right clavicle, lower left rib, and right abdomen for ECG measures (Bush et al., 2016). The ECG and impedance waveforms collected during the DCP yielded measures of HR, RSA, PEP, and respiratory rate (RR). Data were acquired using Mindware Technologies, LTD hardware (www.mindwaretech.com); continuous ECG, Zo (basal impedance), and d*Z*/d*t* (first derivative of the impedance signal) waveforms were recorded. A 4-milliamp AC current at 100 Hz was passed through the two current electrodes; Zo and dZ/d*t* signals were acquired from the two voltage recording electrodes.

The DCP was designed to elicit ANS responses to developmentally-appropriate challenges across different domains: cognitive, physical, and socioemotional and a comparison resting state (Table 2.1). After the electrodes were in place for five minutes, we started the DCP. The DCPs include age-appropriate resting activities with 18-month olds listening to a lullaby and 36-month olds listening to a story read aloud. The challenges varied at each age and at 36-months the protocol included task-specific challenges since the children were able to stay engaged for a longer period of time than the 18-month olds. These protocols were adapted from existing standardized protocols (Alkon et al., 2003, 2006, 2011, 2017; Treadwell et al., 2011; Bush et al., 2011; Hagan et al., 2016; Waters et al., 2016; Suurland et al., 2017) and pilot tested to assess the modified protocols were engaging, developmentally-challenging, and tolerable for each age group.

All study protocols and consent forms were approved by the Institutional Review Board of the University of California, San Francisco.

Data Preparation and Scoring

Autonomic nervous system data were filtered, extracted, and then scored using Mindware Technologies, LTD software programs (HRV 3.1.0F and IMP 3.1.0H). RSA indices are calculated using the interbeat intervals on the ECG waveform, respiratory rates derived from the impedance waveform (e.g., d*Z*/d*t* signal), and a bandwidth range setting of 0.15 to 1.04 (Zisner & Beauchaine, 2015). PEP is measured in milliseconds as the time interval between the onset of ventricular depolarization (Q point on the ECG wave) and the onset of left ventricular ejection (B point on the d*Z*/d*t* wave).

Each 30 second interval was scored if there was a minimum of 20 seconds of clean data. (Zisner & Beauchaine, 2016). The task scores were calculated as the mean of several 30-second intervals depending on the task length (Table 2.1). Outliers were children with RSA or PEP scores greater than three standard deviations from the sample mean for each 30-second interval or task. For the 18-month-olds, there were seven outliers at the interval level and five outliers at the task level. For the 36-month-olds, there were 16 outliers at the interval level and three at the task level. After reviewing the raw data of these outliers with an ANS expert on our team, we decided to keep most of them in the analyses because their other scores showed similar results and were consistent with their individual patterns of physiology. We deleted 12 outliers for 36-month olds at the interval level that differed from their other raw data.

For the 18-month data, RSA and PEP reactivity scores were calculated as the mean response across the three challenge tasks minus the mean of the first resting condition. Reactivity scores were calculated for children who had ANS scored data for at least two of the three challenge tasks. As the 36-month-old protocol included task-specific matched control conditions, 36-month-old RSA and PEP reactivity scores were calculated as the mean of the three difference scores. Each difference score was calculated as the challenge minus the corresponding matched control condition response. The receptive vocabulary challenge was not included in these analyses because there was no task-specific matched control condition, and its intent was to “warm up” children to the activity of engaging with the unknown examiner.

Reactivity scores were only calculated for children with scorable data for at least two of the three different challenge/control difference scores. These reactivity scores were used in the analyses for this paper. Therefore, the reactivity scores for the 18-month-old and 36-month-old protocol were calculated differently because of the inclusion of task-specific control conditions in the 36-month-old DCP (Table 2.1).

To calculate a comparable reactivity score to the 18-month score, we also calculated a 36-month reactivity score of the mean of the four challenges minus the first resting condition. We used this second reactivity score for the 36-month olds in post-hoc analyses comparing reactivity scores across the ages.

Data Analysis

SPSS 25 was used to analyze the data and create the figures. Descriptive statistics were calculated for all of the variables and histograms were created for RSA and PEP resting, challenge, and reactivity scores to show the distribution of the ANS measures. Pearson correlations were calculated to test the stability of the longitudinal relationship for each RSA and PEP measure between 18- and 36-months and scatterplots were created to visualize these associations. Matched-paired t-tests were used to evaluate the continuity of RSA and PEP resting, challenge, and reactivity mean scores between 18- and 36-months of age respectively.

Results

The mothers' mean age at the time of delivery was 38 years of age (range 18-43; SD = 5.8; N = 180). Sixty-eight percent of the mothers were married or had a partner and 54% were multiparous. Thirty-one percent of the mothers had a high school education or less, 50% had some college or vocational training, and 19% had earned a college degree. The families' median annual household income was $19,000, ranging from $0 to $98,000 with the majority of the sample falling 100% below the federal poverty level (FPL) at study enrollment. Eight-five percent of the mothers identified their ethnic or racial backgrounds as 39% African American, 31% Latina, 15% Caucasian, 2% Asian, and 13% other or multiracial. The mother's cesarean rate was 28%, and infants' average gestational age at birth was 39.6 (*SD* = 1.4) weeks.

Children included in these analyses were 19.0 months old (*SD* = 1.3) on average, at the time of their 18-month-old visit (*n* = 134), and were 38.7 months (*SD* = 3.4) at the time of their 36-month-old visit (*n* = 102); 53% of participants at both visit points were girls. Based on the parent's classification of their child's race, the participants included in the 18-month-old visit analysis were 32% White, 14% were Black, and 54% Other (e.g. Asian, Native American, mixed race); 42% were classified as Hispanic. Among children included in 36-month-old visit analyses, 36% were White, 12% were Black, and 52% were Other, with 33% of the total further classified as Hispanic. The mean household federal poverty level was less than 200% at the 18-month and 36-month-old visits.

Children's RSA and PEP resting, challenge, and reactivity at 18- and 36-months are presented in Table 2.2. Overall, RSA and PEP resting, challenge, and reactivity measures showed normal distributions at both age timepoints. The mean RSA and PEP resting and challenge scores were lower at 18-months compared to 36-months of age. The mean RSA and PEP reactivity scores were positive at 18 months and negative at 36-months of age.

The mean levels of PNS and SNS activity across the DCP at 18- and 36-months of age are depicted in a line plot chart in Figure 2.1. These figures describe the change in response to the resting and challenge conditions at 18- and 36-months of age. At 18-months, RSA levels were lowest during the lullabies and lemon juice challenge and highest for the emotion challenge (infant cry) (Table 2.1). PEP was lowest for the first lullaby and highest for the startle challenge (e.g., jack-in-the-box). At 36-months, RSA was highest during the first resting period, reading a book, and the lowest for the taste challenge (e.g., lemon juice on the tongue). PEP was highest for the task–specific control of picture identification and lowest for the cognitive challenge (e.g., repeating numbers). At 18-months of age, the largest changes in reactivity were from the taste challenge exhibiting PNS withdrawal and the emotion challenge exhibiting SNS activation. At 36-months of age, the largest changes in reactivity were also from the taste challenge with PNS withdrawal and the cognitive challenge with SNS activation.

RSA and PEP resting, challenge, and reactivity measures from 18- to 36-months of age are shown to be significantly positively, moderately correlated (see Figure 2.2). Findings for specific variables included: RSA resting (r = 0.3, n = 96), PEP resting (r = 0.6, n = 90), RSA challenge (r = 0.4, n = 92), and PEP challenge (r = 0.6, n = 87). In addition, PEP means under challenge conditions at 18-months were significantly positively, moderately correlated with PEP during rest at 36-months (r = 0.6). RSA means under challenge conditions at 18-months were significantly positively, moderately correlated with RSA during rest at 36-months (r = 0.3). PEP during rest at 18-months was significantly, weakly, negatively correlated with RSA during rest at 36-months (r = -0.2). RSA reactivity (r = 0.01, n = 91) and PEP reactivity (r = 0.02, n = 84) were not correlated between these time points.

The continuity results (Table 2.3) showed that there were statistically significant increases in sample mean RSA and PEP resting, challenge, and reactivity values between 18- and 36-months of age, within ANS measure. The greatest mean changes were in PEP resting (M = 6.5, SD = 6.0) and PEP challenge (M = 5.3, SD = 5.8).

Discussion

This study revealed novel information about the distribution, stability, and continuity of respiratory sinus arrhythmia (RSA) and preejection period (PEP) rest, challenge, and reactivity measures from 18- to 36-months of age in an under-represented, low-income, predominantly minority sample. RSA and PEP rest, challenge, and reactivity measures displayed normal distributions with little evidence of skewness or kurtosis at 18- and 36-months of age. Next, there was moderate stability in the PNS and SNS resting and challenge responses within individuals over time but not in reactivity measures. There was a lack of continuity across the ages with significant mean increases of RSA and PEP rest, challenge, and reactivity from 18- to 36-months.

Consistent with previous research of children in similar age groups, RSA and PEP displayed a normal distribution, reflecting individual differences and a range of ANS activity across children at each age. There were approximately equal numbers of children with positive and negative RSA or PEP reactivity scores. Figure 2.1 illustrates that, on average, the children at 18-months may have begun the protocol stressed by the strange situation of having electrodes placed on their chest and starting an unfamiliar protocol. On the other hand, at 36-months of age the children appeared able to relax at the start of the protocol when an adult read a story aloud to them. In addition, the mean responses showed that many of the preschool-age children were able to return to their resting levels after the challenges were over and the adult read another story aloud to them. This is the first study that we are aware of to report on both RSA and PEP resting, challenge, and reactivity measures at 18-months of age. Developmentally, it is challenging to engage toddlers in specific tasks and to sustain their attention to measure resting states. Thus, this study contributes new knowledge at this age and compares their ANS responsivity at 36 months of age.

In previous studies, RSA resting, PEP resting, and RSA challenge levels in infants and children have consistently shown stability over time; whereas, PEP challenge, RSA reactivity, and PEP reactivity have not shown stability (Hinnant et al., 2017). The results of this study align with Hinnant and colleagues' (2017) findings of moderate stability in 8- to 10-year-olds for RSA resting, PEP resting, and RSA challenge, but our study findings differed in our finding of moderate stability in 18- to 36- month-olds in PEP challenge measures. However, RSA reactivity was not stable between these age points, consistent with Hinnant and colleagues' (2017) 8- to 10-year-old findings and Alkon and colleagues' (2011) findings in a cohort of primarily Latino children from six months to five years of age. In this study, PEP reactivity also did not show stability between 18- and 36-months. This lack of stability in the PNS and SNS reactivity indicates the potential for plasticity in stress physiology during the early childhood period. Plasticity indicates individual changes in a child's stress responsivity from one time point to another (within-person variability) or changes over time in the stress response from person to person (between-person variability) (Hinnant et al., 2017). Our findings and others' support the theories of individual differences, such as biological sensitivity to context (BSC), in the stress response (Boyce & Ellis, 2005) as there are a range of ANS responses at each age group.

Since this was a longitudinal study, we needed to design an ANS protocol with similar challenges for each developmental domain but adjust the type and length of tasks (Table 2.1). At 36-months of age we were able to include task specific challenges so we calculated two reactivity scores. Similar to the 18-month reactivity score, we subtracted the first resting condition from the mean of the four challenge conditions. The 36-month reactivity score, not comparable to 18-months, was the mean of three reactivity scores computed as the challenge-specific control minus the challenge. Specifically, within measure, RSA and PEP reactivity are not correlated from 18- to 36-months of age no matter whether the 36-month-old reactivities were calculated when controlling for or not controlling for task specific responses.

The statistically significant mean level changes of RSA and PEP resting, challenge, and reactivity from 18- to 36-months of age are indicative of developmental changes or a lack of continuity expected over time. Gatzke-Kopp & Ram (2018), Hinnant et al. (2017), Conradt et al. (2014), and Alkon et al. (2011) found more PNS withdrawal compared to rest for older children compared to younger children's longitudinal cohort samples. PEP resting and PEP challenge had larger mean change compared to the other measures which may suggest that the SNS response becomes more responsive over time. From 18- to 36-months of age, the children showed greater physiological responsiveness to the challenges as evidenced by a negative RSA and PEP reactivity measures (change in RSA reactivity = -0.6, change in PEP reactivity = -0.9). This finding suggests that older children may be more biologically responsive or sensitive to the challenges presented during the protocol or that they were more engaged in the ANS protocol compared to the 18-month olds. Overall, the mean changes from 18- to 36-months of age are consistent with other studies of continuity from 6-months to 10-years of age (Alkon et al., 2006 & 2011; Bar-Haim, Marshall, & Fox, 2000; Bornstein & Suess, 2000; Calkins & Keane, 2004). Other studies also support similar findings of increasing reactivity as the child ages (Alkon et al., 2006 & 2014). This is the first study that shows mean RSA and PEP changes from 18- to 36-months of age.

Although this study contributed new findings to the field of ANS function in young children, there are several limitations to consider. These findings are limited to two time points of repeated measures. However, these data are described in light of two complementary constructs, stability and continuity, that are important to report in a longitudinal developmental study and only requires two waves of longitudinal data. While the SEED study does have RSA and PEP measurements from these same children at age 6 months, the 6-month ANS protocol utilized an attachment-based Still Face Paradigm (SFP), rather than a developmentally challenging protocol like the 18- and 36-month protocols, and funding delays led to only half the cohort being assessed for ANS. Thus, our analyses conducted here were not appropriate to model starting during infancy. We also note that the challenges in the DCP were not randomized and order of challenges may affect physiologic responsivity. Impedance cardiography is sensitive to movement and respiratory artifact (Bush et al., 2011; Zisner & Beauchaine, 2016). However, these ANS measures (RSA and PEP) offer an inexpensive, noninvasive estimation of central nervous system function as compared to EEG or fMRI studies.

Future Directions. We recommend the use of standardized, valid measures and methods for the assessment of ANS during resting and challenge conditions. For example, a protocol that assesses ANS responsivity including reactivity should be holistic and include a range of domains, such as the physical, emotional, cognitive, and social, and these protocols should be repeated in the same children across time. Administration of the 18-month-old protocol could be revised to illict a better measure of rest as many children were not relaxed at the start of the protocol. Additionally, calculating reactivity scores separately by domain revealed that the greatest ANS reactivity was found with the physical challenge. The children had significant SNS activation and PNS withdrawal with the lemon taste challenge. Future analyses of this challenge-specific reactivity may be helpful for investigating how children at a certain age responds to a specific developmental domain and possible environmental factors that may affect the response. This challenge may also have specific utility for eliciting reactivity in situations where it is not possible to administer the entire challenge protocol or a study includes specific outcomes related to satiation or taste reactivity. Stability and continuity are important terms to define and report in a consistent manner across studies, especially longitudinal studies (Bornstein, Putick, & Esposito, 2017). Other researchers suggest that RSA and PEP measures be collected in conjunction with neuroimaging methods (such as fMRI, EEG, or PET) to assess neural correlates of autonomic functioning (Zisner & Beauchaine, 2016). Adding measures of genetic predisposition, environmental risk, and protective factors that may be associated with ANS reactivity can tease out the interplay of endogenous and exogenous factors.

Conclusion. A child's neurobiological circuitry develops rapidly from birth to five years of age (Shonkoff et al., 2014). Understanding the measurable indicators that can affect the trajectory of these neurobiological pathways can reveal important information that may inform interventions to affect change at earlier and more developmentally-sensitive time points. RSA and PEP are valuable measures to assess changes in autonomic nervous system activity over time, with implications for understanding concepts such as biological embedding of early childhood experiences, biological self-regulation, and child adjustment, all of which can have important consequences for the health and wellness of an individual over their lifespan.

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Table 2.1 Developmental Challenge Protocols (DCP) at 18- and 36-Months of Age

18-month-old DCP			
Task #	**Task**	**Time**	**Task Type**
T1	Lullaby #1	1 min	Rest
T2	Jack in the Box toy	1 min	Cognitive (startle)
T3	Lemon juice on tongue	30 sec	Physical (sensory)
T4	Sick infant cry recording	30 sec	Emotion (socioemotional)
T5	Lullaby #2	1 min	Rest
	Total	**4 min**	

36-month-old DCP			
Task #	**Task**	**Time**	**Task Type**
T1	Calm story read aloud #1	2 min	Rest
T2	Picture identification	90 sec	Cognitive (receptive vocabulary)
T3	Repeat after me	1 min	Control condition for cognitive task
T4	Picture naming	90 sec	Cognitive (expressive vocabulary)
T5	Water drink	30 sec	Control condition for physical task
T6	Lemon juice on tongue	30 sec	Physical (sensory)
T7	Calm video	2 min	Control condition for emotion task
T8	Scary video	2 min	Emotion (fear)
T9	Calm story read aloud #2	2 min	Rest
	Total	**13 min**	

Table 2.2 Distribution of RSA and PEP Resting, Challenge, and Reactivity at 18- and 36-Months of Age

	RSA resting		**RSA challenge**		**RSA reactivity**		**PEP resting**		**PEP challenge**		**PEP reactivity**	
Months	**18**	**36**	**18**	**36**	**18**	**36**	**18**	**36**	**18**	**36**	**18**	**36**
N	134	102	133	98	133	97	127	100	128	96	124	96
Mean	4.99	6.53	5.28	6.05	0.27	-0.36	80.48	87.28	81.12	86.41	0.59	-0.05
SD	1.22	1.08	1.04	1.01	0.73	0.49	6.01	6.79	6.03	6.66	2.33	1.78
Range	2.12, 8.06	4.02, 8.87	2.86, 7.97	3.81, 8.41	-1.36, 3.19	-2.66, 1.37	68, 95	70.5, 101.5	67.83, 94.67	70.89, 101.42	-6.33, 7.17	-6.08, 7.75
Skewness	0.20 (0.21)	0.00 (0.24)	0.36 (0.21)	0.20 (0.24)	0.56 (0.21)	-0.75 (0.25)	-0.06 (0.22)	-0.32 (0.24)	-0.04 (0.21)	-0.17 (0.25)	0.11 (0.22)	0.82 (0.25)
Kurtosis	0.18 (0.42)$^{\#}$	-0.29 (0.47)$^{\#}$	0.03 (0.42)$^{\#}$	-0.04 (0.48)*	1.87 (0.42)	5.29 (0.49)	-0.40 (0.43)*	-0.28 (0.48)$^{\#}$	-0.68 (0.43)$^{\#}$	-0.45 (0.49)*	0.30 (0.43)*	4.54 (0.49)

RSA: respiratory sinus arrhythmia; PEP: preejection period
Shape of distribution: * = peaked, $^{\#}$ = flat

Figure 2.1 Mean Levels of Paraympathetic and Sympathetic Activity Across the 18- and 36-Month-Olds Developmental Challenges Protocol (DCP)

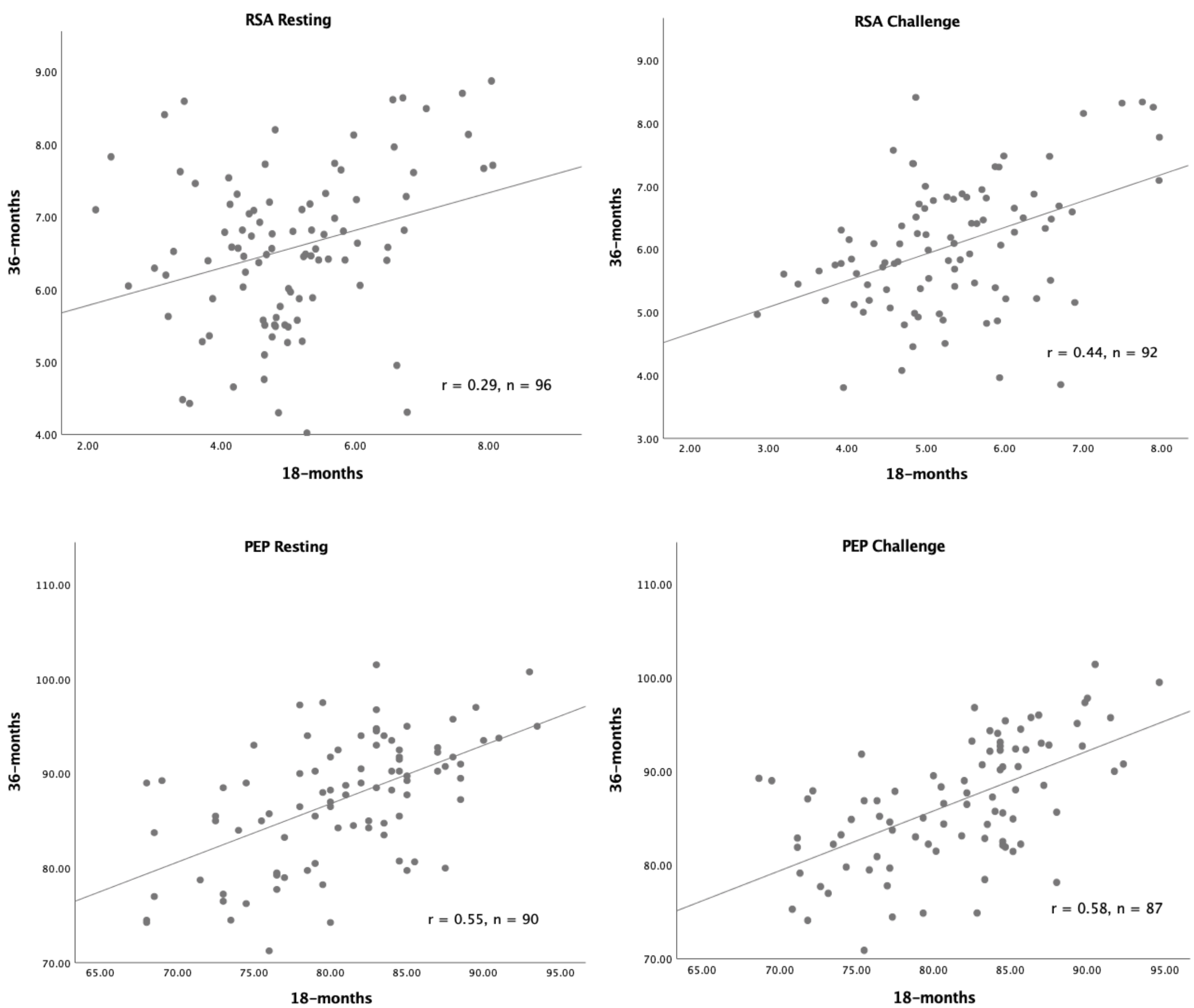

Figure 2.2. Correlations of RSA and PEP Resting and Challenge from 18-Months to 36-Months of Age (n = 84-96)

Table 2.3 Mean Change of RSA and PEP Resting, Challenge, and Reactivity from 18- to 36-Months of Age

	Mean Change	SD	t-statistic	df	p-value
RSA resting	1.5	1.4	10.8	95	0.000
RSA challenge	0.8	1.1	6.4	91	0.000
RSA reactivity	-0.6	0.9	-6.7	90	0.000
PEP resting	6.5	6.0	10.4	89	0.000
PEP challenge	5.3	5.8	8.5	86	0.000
PEP reactivity	-0.9	2.9	-2.9	83	0.005

SD = standard deviation; RSA: respiratory sinus arrhythmia; PEP: preejection period

Chapter Three: Second Manuscript

Autonomic Nervous System Reactivity in Early Childhood: Developmental Patterns from 18- to 36-Months of Age

Abstract

Cardiac measurements of autonomic nervous system (ANS) activity, respiratory sinus arrhythmia (RSA) and preejection period (PEP), measured during resting and challenging conditions, reflect an individual's physiologic response to stressors at one point in time and possibly across time. This study's aims were to combine RSA and PEP reactivity based on activation or withdrawal of the parasympathetic (PNS) or sympathetic nervous system (SNS) and classify according to Berntson and colleagues' four ANS reactivity profiles, and to assess the stability of membership within the profiles. At 18-months (n = 134) and 36-months (n = 102) of age, children completed a developmentally challenging protocol that simultaneously assessed their RSA and PEP under resting and challenge conditions. Four ANS profiles were created by dichotomizing reactivity, mean challenge minus rest, as positive or negative reactivity. The majority of study participants were girls, racially and ethnically diverse, and lived in households 200% below the federal poverty level. Results showed that 46% of 18-month-olds were categorized as reciprocal PNS activation/SNS not activated and 49% of 36-month-olds were reciprocal SNS activation/PNS withdrawal. There was a statistically significant shift from 18- to 36-months in the children's (n = 80) distributions across the four profiles. Overall, only 21% of children (n = 17) had the same ANS reactivity profile at 18- and 36-months of age; however, 58% of the children with the reciprocal SNS activation/PNS withdrawal profile at 18-months remained in that profile at 36-months. The significant shift of children to different profiles coupled with the moderate stability of one profile, reciprocal SNS activation/PNS withdrawal,

supports the need for research of the individual and environmental factors that predict developmental changes versus stability of ANS profiles over time.

Keywords: autonomic nervous system, respiratory sinus arrhythmia, preejection period, sympathetic nervous system, parasympathetic nervous system, early childhood, psychobiological

Introduction

Adverse events and conditions experienced during childhood can have a profound impact on physical, mental, and behavioral health across the lifespan (Felitti et al., 1998; Burke et al., 2011). Children can be affected by adverse environmental experiences occurring as early as the prenatal period (Bush et al., 2017; Weiss et al., 2007). However, not all children are equally susceptible to adverse experiences (Boyce & Ellis, 2005). The autonomic nervous system (ANS) is key in regulating the physiological stress response, and assessment of ANS functioning allows for study of individual differences in stress response. Several studies have found associations between ANS responsivity and childhood health, learning, and behavioral outcomes (Shonkoff et al., 2014). These findings inspire further study of ANS responsivity during early childhood, in the hope that research findings may inform clinical interventions to mitigate the impact of adverse experiences on children.

The autonomic nervous system is comprised of two branches, the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). The PNS is the "rest and restorative" system that, when activated, promotes a lower heart rate and relaxed state. The SNS is the "fight or flight" system that, when activated, accelerates the heart rate as if to prepare the individual to take action (Berntson, Quigley, & Lozano, 2007). The PNS originates from the nuclei within the brainstem and from sacral cord segments and the SNS arises from the thoracolumbar system (Bernston, Quigley, & Lozano, 2007). Postganglionic neurons from both systems fire messages to target organs. Postganglionic PNS fibers employ acetylcholine and typically react on muscarinic receptors (i.e., of the heart). Postganglionic SNS fibers primarily employ norepinephrine which innervates either alpha adrenergic or beta-adrenergic receptors (i.e., beta-1 adrenergic ($ß_1$) of the heart). While there are exceptions to these patterns, this important differentiation allows for study of ANS reactivity based on cardiac measures.

Impedance cardiography allows for non-invasive assessment of a range of cardiac measures and can be used in a research setting to monitor specific cardiac markers of PNS and SNS activity during a defined psychophysiological event (e.g. a stress-inducing protocol). The cardiac measurement that most closely reflects PNS activity is respiratory sinus arrhythmia (RSA) and SNS activity is preejection period (PEP). Reactivity is defined as the change in an individual's physiologic response from a challenge to a resting state (Alkon et al., 2017), and is quantified in terms of difference scores (i.e. difference in measures between challenge and resting conditions) (Matthews, 1986). Reactivity has different meaning for PNS and SNS measures, with four key categories defined as follows (Alkon et al., 2017):

- *High RSA reactivity* (i.e., negative RSA difference score) indicates PNS withdrawal (withdrawal of the vagus nerve) during the challenges compared to the resting state.
- *High PEP reactivity* (i.e., negative PEP difference score; shortening of the PEP during the challenges compared to rest) indicates more SNS activation (increased "fight or flight" impulse) during the challenges compared to the resting state.
- *Low RSA reactivity* (i.e., positive RSA difference score) indicates more PNS activation (i.e., more vagal input, or promotion of a relaxed state) during the challenge compared to the resting state.
- *Low PEP reactivity* (i.e., positive PEP difference score; lengthening of the PEP during the challenges compared to rest) indicates SNS not activated during the challenge compared to the resting state.

Historically, the autonomic nervous system (ANS) response was conceptualized as a single continuum, with sympathetic activation (“fight or flight” impulse) on one end and parasympathetic activation (relaxation state) on the other (Berntson et al., 2007). The concept of autonomic space challenges this notion, positing that characterization of autonomic response, requires, at a minimum, a two-dimensional model, with SNS activity and PNS activity treated as separate axes within this space (Berntson et al., 1991). Working within this two-axis model, Berntson et al. (1991) proposed modes of autonomic control that fall into six categories: reciprocal (reciprocal parasympathetic activation, reciprocal sympathetic activation), nonreciprocal (coactivation or coinhibition), or uncoupled (uncoupled parasympathetic changes, uncoupled sympathetic changes) activity. Because unilateral (i.e. uncoupled) activation profiles are not common in children, a four-profile categorization has been applied most commonly in children (Salomon et al., 2000; Alkon et al., 2003). These four profiles are conceptualized as follows:

- *Coactivation*: the SNS and PNS are both activated in challenge (versus rest) conditions, indicated by a positive RSA reactivity score and a negative PEP reactivity score. An increased “fight or flight” impulse occurs simultaneously with promotion of a relaxed state.
- *Coinhibition:* the SNS and PNS are both inhibited in challenge conditions, indicated by a negative RSA reactivity score and a positive PEP reactivity score. A decreased “fight or flight” impulse occurs along with withdrawal from a relaxed state.
- *Reciprocal parasympathetic (PNS) activation/sympathetic (SNS) not activated:* the SNS is inhibited and the PNS is activated in challenge conditions, indicated by a positive RSA

reactivity score and a positive PEP reactivity score. Promotion of a relaxed state occurs with decreased "fight or flight" impulse.

- *Reciprocal sympathetic (SNS) activation/parasympathetic (PNS) withdrawal (aka classic reactivity):* the SNS is activated and the PNS is inhibited in challenge conditions, indicated by a negative RSA reactivity score and a negative PEP reactivity score. Increased "fight or flight" impulse occurs along with withdrawal from a relaxed state.

Table 3.1 provides an overall conceptualization of how the ANS profiles are categorized by RSA and PEP reactivity and PNS and SNS activity. This study aims to combine RSA and PEP reactivity based on activation or withdrawal of the parasympathetic or sympathetic nervous system and classify according to Berntson and colleagues' four ANS reactivity profiles, and to assess the stability of membership within the profiles.

Review of the Literature. Since the early 2000s, a number of researchers have examined autonomic reactivity in samples of children and adolescents and have assigned reactivity profiles defined by both PNS and SNS activity (see Figure 3.1). Though exact protocols have differed across studies, all the studies reviewed below have assessed children's cardiac activity during both age-appropriate challenge and resting conditions. The general aims of the literature to date have been to characterize ANS reactivity patterns at different ages, to explore whether adverse early experiences (e.g. family conflict) are associated with children's reactivity patterns, and to describe behavioral characteristics of children in different profiles. The studies reviewed below have employed two main systems to define profiles. Several have followed the four-profile version of the Berntson et al. (1991) categories as described above. Several recent studies assigned ANS profiles consistent with a newer Adaptive Calibration Model (ACM), (Ellis et al., 2017; Kolacz et al., 2016; Del Giudice et al., 2012). The ACM proposed four patterns (sensitive, buffered, vigilant, and unemotional); the sensitive pattern displays high PNS and moderate SNS responsivity, the buffered pattern has more PNS activity compared to SNS activity, the vigilant pattern has high SNS and low PNS responsivity, and the unemotional pattern has low PNS and SNS responsivity. These ACM patterns can be tangentially matched with the Berntson et al. (1991) ANS profiles to assist with a holistic interpretation of the studies thus far that have looked at the combined effect of PNS and SNS activity.

Solomon, Matthews, and Allen in 2000 assessed ANS reactivity and then classified their responses according to Berntson et al. (1991) four ANS profiles. Of their sample of 201 participants, 48% were Black, 49% were female, and between 8-17 years of age. They collected measurements of PEP and RSA while the participants underwent a protocol consisting of an initial 10-minute rest period, then they completed three tasks (reaction time, interview, and mirror) with an 8-minute rest period in between the tasks and ended with a final 10-minute rest period. The largest group of participants showed a reciprocal SNS activation/PNS withdrawal pattern. They did not find any difference of the profiles by age or gender.

Alkon et al. (2003) reported on three cross-sectional studies comprising a total of 273 children of mostly White, college graduate mothers with a family income of $60,000 or above, aged 3-8 years in three different studies and settings (e.g., child's home, child care center, or research laboratory). A developmentally appropriate challenges protocol was administered that offered a 2-minute relaxing story read aloud at the beginning and end of the protocol as the resting condition and the challenge tasks elicited four different domains (social, cognitive, physical, and emotional). The children's RSA and PEP reactivity scores were dichotomized as positive or negative and categorized into the four profiles (coactivation, coinhibition, reciprocal

PNS activation/SNS not activated, and reciprocal SNS activation/PNS withdrawal) proposed by Berntson et al. in 1991. The 3- to 4-year-olds ANS profile distributions were: coactivation (16%), coinhibition (36%), reciprocal PNS activation/SNS not activated (16%) and reciprocal SNS activation/PNS withdrawal (32%). The 5- and 6-year-olds' profile distributions were: coactivation (16%), coinhibition (37%), reciprocal PNS activation/SNS not activated (17%), and reciprocal SNS activation/PNS withdrawal (30%). The 7- and 8-year-olds' profile distributions were: coactivation (13%), coinhibition (43%), reciprocal PNS activation/SNS not activated (30%), and reciprocal SNS activation/PNS withdrawal (14%). These results revealed that coactivation is the least frequent group (16%) and the percent did not change with age. Coinhibition and reciprocal PNS activation/SNS not activated were more prevalent profiles as the children got older. Reciprocal SNS activation/PNS withdrawal decreased in prevalence with age, which may signify fewer children with a classic reactivity profile at school-age.

Pearson et al. (2005) conducted a pilot, cross-sectional study (N = 19) of four to nine-year olds with homozygous sickle cell disease and assessed their ANS reactivity, clinical severity, family stressors, and mental health. Their mean age was 7.4 years and they mostly had parents who lived together with a GED education or above. The developmental challenges protocol was similar to the Alkon et al. 2003, 2011, and 2017 studies. Their ANS reactivity was categorized according to the Bernston et al. (1991) profiles and they found that children with high levels of externalizing were more likely to have a classic reactivity profile. There were no other significant differences across profiles for internalizing disorders; however, they did find that children with greater PNS withdrawal had more severe disease and those with greater SNS activation had more externalizing behaviors.

El-Sheikh et al. (2009) conducted three cross-sectional studies to look at the relationship between marital conflict and child adjustment with ANS reactivity as a moderator. The first study of 176 children (mean age = 8.69 years), 56% of whom were girls, of all socioeconomic levels and the majority were African American, had a protocol that began with 10-minutes of conversational speaking, an additional 2-minutes to acclimate to the laboratory setting, followed by 3-minutes of baseline assessment, then a challenge of listening to a 3-minute audio-taped inter-adult conflict, a recovery period of 12-minutes, and ended with the star-tracing task. Researchers used RSA as a measure of PNS activity and SCL as a measure of SNS activity. The second study of 251 children with a mean age of 8.23 years, 51% of whom were girls, 64% European American and 36% African American, had mostly families with two parents in the home together with a median family income of $35-50,000. The third study had 150 children, even split of girls and boys, with a mean age of 9.27 years (SD = 1.95), 67% European American with a median family income of $35-50,000. Across all three studies, they repeated the protocol (study two only had a six-minute recovery and study three only had six-minutes to acclimate, three-minutes for baseline, and five-minutes for recovery) and found similar results. Using the Berntson et al. (1991) ANS reactivity profiles, they found that marital conflict predicted delinquency in children who were grouped in the coinhibition profile. The reciprocal PNS activation/SNS not activated profile appeared to be a protective factor in this relationship. Additionally, children with a coactivation profile were associated with more behavioral problems at higher levels of marital conflict.

Alkon et al. (2011) looked at the four profiles longitudinally in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort study, a sample of mostly Latino children (N = 378), born in Mexico, 62% living at or below the federal poverty level, of young Latinx mothers (< 30 years of age), when the children were 6-, 12-, 42-, and 60- months of age. The developmental challenges protocol was similar to the Alkon et al., 2003 study, modified slightly since it was administered primarily in Spanish and the challenges were modified for the infants. From infancy to 5-years of age, the profiles significantly differed, and the reciprocal SNS activation/PNS withdrawal profile was the most prevalent by 42-months of age. The profile that had the largest increase in prevalence from 6-months to 60-months was coinhibition; from 6% to 27%.

Del Giudice, Hinnant, Ellis, & El-Sheikh (2012) used the Adaptive Calibration Model (ACM) as their theoretical framework to assess the combined effect of the PNS and SNS branches in a cross-sectional study of 256, 8-10-year-olds (53% boys). The mean age of the children was 9.4 years (most children were pre-pubertal), 64% European American and 36% African American with the majority of family incomes between $20,000 -75,000. The researchers measured RSA and skin conductance level (SCL) with a protocol that included three minutes to adapt to the laboratory, three minutes of the child sitting quietly for a baseline measurement, and three minutes of the star tracing task. Using latent class profile analyses, they found four patterns: sensitive (high resting PNS and moderate resting SNS), buffered (moderate resting PNS and low resting SNS), vigilant (low resting PNS and high resting SNS), and unemotional (low resting PNS and SNS). The largest group was buffered (45%), then sensitive (27%), followed by unemotional (18%), and vigilant (10%). They found increased negative family relationships and low levels of family warmth/predictability increased odds of being in the vigilant or unemotional pattern, which are high stress classes. The sensitive and unemotional pattern had more variability than the buffered or vigilant pattern, but the unemotional pattern had the most variability of all the patterns.

Quas et al. (2014) conducted a cross-sectional analysis of four independent studies totaling 664 children from 4 to 14 years of age to understand the patterns of SNS, PNS, and HPA-axis responsivity and how these three measures could be categorized into patterns. These children were 50% girls, >50% college-educated parents, and median family income of $50,000-$100,000. The protocol consisted of four challenges, one from each of the four domains of development (social, cognitive, sensory, and emotional), lasting 1-2 minutes each, and preceded by a motor-activity matched baseline activity. A latent profile analysis revealed 6 patterns of ANS reactivity: 1) moderate, 2) parasympathetic-specific, 3) anticipatory arousal, 4) multisystem, 5) Hypothalamic Pituitary Adrenal (HPA) axis-specific, and 6) underarousal. The most common pattern was the moderately reactive group. The parasympathetic-specific group was represented throughout all of the age groups suggesting these children were reactive by having a predominately parasympathetic withdrawal response. The anticipatory arousal profile, represented throughout the ages except for the 11-year-olds, could be reflective of poor and chronic dysregulation due to accrued allostatic load.

Kolacz et al. (2016) also studied the ACM patterns but in relation to parent-reported temperament and baseline physiological activity among preschool-aged children within a longitudinal study. This cross-section of 36-month-olds (N = 101), 49% female, 59% African American, with a median family income of $46,975 reported on RSA, salivary alpha amylase (sAA), and cortisol. Cortisol and sAA were collected within five-minutes time of arriving in the waiting room and RSA was collected after a two-minute resting period. Their resting RSA M = 5.5, SD = 1.4 and a cluster analysis revealed four ACM profiles: sensitive (n = 17), buffered (n = 45), vigilant-high HPA (low PNS, moderate SNS, and high HPA) (n = 15), and vigilant-low HPA (high SNS, low PNS, and low HPA) (n = 24) with no evidence found for the unemotional profile. They found that child's biological sex was unrelated to the profiles, African American children had higher odds of being in the sensitive or vigilant-high or vigilant low profiles compared to the buffered group, and the buffered group were significantly lower in negative affectivity than those in the vigilant-low HPA group.

Gaps

Overall, these studies have found that the classic reactivity profile is most prevalent after 36-months of age and before then, the reciprocal PNS activation/SNS not activated the most prevalent. The coactivation profile does not appear to be relevant in childhood; however, the coinhibition profile is consistently present throughout childhood. To date, there are studies on ANS reactivity that do not acquire measurements from a single organ (i.e., the heart) (Beauchaine, 2009). About 33% of the reviewed studies used SCL (derived from the skin) or sAA (derived from an accessory organ) as their proximal measurement of SNS activity. These indices may be measuring too distal from the initial SNS activity. Therefore, not collecting ANS reactivity measurements from a single organ can decrease the validity and reliability of the study. Repeated cardiac measurements of ANS reactivity in children younger than three years of age are rarely collected, as Alkon et al. 2011 study has been the only one to date. RSA and PEP should be measured in an individual over time so as to understand their stability and consistency. Furthermore, RSA and PEP has not been previously studied in 18-month-old children. Up until this point, there is no knowledge of RSA and PEP being measured in children between the ages of 12-36 months of age. This is a sensitive period of development and there would be great benefit to knowing about the responsivity of the ANS during this time.

Aims/Hypotheses

The two aims of this paper are to: describe the proportion of children at 18 and 36 months of age in the four ANS reactivity profiles (i.e., 1) coactivation, 2) coinhibition, 3) reciprocal PNS activation/SNS not activated, 4) reciprocal SNS activation/PNS withdrawal); and, assess the stability of membership within the four ANS reactivity profiles from 18 months to 36 months of age. The hypotheses are: a) the distribution of children in each of the four ANS reactivity profiles will differ at 18 and 36 months of age, b) the proportion of children with the "classic" reactivity, or the reciprocal sympathetic activation/PNS withdrawal profile, will be greater at 36 months than at 18 months of age, and c) there will be stability in the distribution of RSA and PEP reactivity from 18- to 36- months of age, d) there will be a lack of stability amongst the ANS profiles from 18- to 36-months of age.

Methods

This cohort study is part of a larger birth cohort study, the Stress, Eating, and Early Development (SEED) study, which was a continuation of the Maternal Adiposity, Metabolism, And Stress (MAMAS) study. The MAMAS study was a non-randomized control trial designed to examine the effects of a mindfulness-based stress reduction and healthy lifestyle intervention to reduce excessive gestational weight gain among pregnant women (Bush et al., 2017). The SEED study followed the children of MAMAS mothers from birth through age five, investigating the impact of prenatal and early childhood stress and eating habits on the children's behavioral, physiologic, and anthropometric development. This paper describes and analyzes the ANS data collected at the 18-month and 36-month visits.

The children of the MAMAS study were born of mothers of low to middle income and overweight or above. All of the 215 mothers of the MAMAS study were invited to enroll themselves and their newborns in the follow-up SEED study at delivery. In total, 162 mother-child dyads (84%) enrolled in SEED, of whom 134 children (74% of the enrolled delivery sample) and 102 children (57% of the delivery sample) participated fully at 18-month and 36-month study visits, respectively.

Data Collection

For both the 18- and 36-month visits, a trained research assistant (RA) met with the mother and child in the family's home and the dyad were together during the study visit. During these visits, the research assistant administered multiple questionnaires with the mother, took anthropometric measurements of the child, and conducted a standardized, age-specific Developmental Challenges Protocol (DCP) with the child while simultaneously monitoring their ANS activity (RSA and PEP). The measures from the larger MAMAS and SEED studies will not be reported in this study.

For the 18-month visit, the child sat on the mother's lap facing the RA during the protocol, but for the 36-month visit the child sat independently while the mother was in the room but not within sight of the child. The research assistant placed four spot electrodes on the child's neck and trunk to collect impedance and respiratory measures and three spot electrodes were placed on the right clavicle, lower left rib, and right abdomen for ECG measures (Bush et al., 2016). The ECG and impedance waveforms collected during the DCP yielded measures of HR, RSA, PEP, and respiratory rate (RR). Data were acquired using Mindware Technologies, LTD hardware (www.mindwaretech.com); continuous ECG, Zo (basal impedance), and d*Z*/d*t* (first derivative of the impedance signal) waveforms were recorded. A 4-milliamp AC current at 100 Hz was passed through the two current electrodes; Zo and dZ/d*t* signals were acquired from the two voltage recording electrodes.

The DCP was designed to elicit ANS responses to developmentally-appropriate challenges across different domains: cognitive, physical, and socioemotional and a comparison resting state (Table 2.1). After the electrodes were in place for five minutes, we started the DCP. The DCPs include age-appropriate resting activities with 18-month-olds listening to a lullaby and 36-month-olds listening to a story read aloud. The challenges varied at each age and at 36-months the protocol included task-specific challenges since the children were able to stay engaged for a longer period of time than the 18-month-olds. These protocols were adapted from existing standardized protocols (Alkon et al., 2003, 2006, 2011, 2017; Treadwell et al., 2011; Bush et al., 2011; Hagan et al., 2016; Waters et al., 2016; Suurland et al., 2017) and pilot tested to assess the modified protocols were engaging, developmentally-challenging, and tolerable for each age group.

All study protocols and consent forms were approved by the Institutional Review Board of the University of California, San Francisco.

Data Preparation and Scoring

Autonomic nervous system data were filtered, extracted, and then scored using Mindware Technologies, LTD software programs (HRV 3.1.0F and IMP 3.1.0H). RSA indices are calculated using the interbeat intervals on the ECG waveform, respiratory rates derived from the impedance waveform (e.g., d*Z*/d*t* signal), and a bandwidth range setting of 0.15 to 1.04 (Zisner & Beauchaine, 2015). PEP is measured in milliseconds as the time interval between the onset of ventricular depolarization (Q point on the ECG wave) and the onset of left ventricular ejection (B point on the d*Z*/d*t* wave).

Each 30 second interval was scored if there was a minimum of 20 seconds of clean data. (Zisner & Beauchaine, 2016). The task scores were calculated as the mean of several 30-second intervals depending on the task length (Table 2.1). Outliers were children with RSA or PEP scores greater than three standard deviations from the sample mean for each 30-second interval or task. For the 18-month-olds, there were seven outliers at the interval level and five outliers at the task level. For the 36-month-olds, there were 16 outliers at the interval level and three at the task level. After reviewing the raw data of these outliers with an ANS expert on our team, we decided to keep most of them in the analyses because their other scores showed similar results and were consistent with their individual patterns of physiology. We deleted 12 outliers for 36-month-olds at the interval level that differed from their other raw data.

For the 18-month data, RSA and PEP reactivity scores were calculated as the mean response across the three challenge tasks minus the mean of the first resting condition. Reactivity scores were calculated for children who had ANS scored data for at least two of the three challenge tasks. As the 36-month-old protocol included task-specific matched control conditions, 36-month-old RSA and PEP reactivity scores were calculated as the mean of the three difference scores. Each difference score was calculated as the challenge minus the corresponding matched control condition response. The receptive vocabulary challenge was not included in these analyses because there was no task-specific matched control condition. Reactivity scores were only calculated for children with scorable data for at least two of the three different challenge/control difference scores. These reactivity scores were used in the analyses for this paper. Therefore, the reactivity scores for the 18-month-old and 36-month-old protocol were calculated differently because of the inclusion of task-specific control conditions in the 36-month-old DCP (Table 2.1). To calculate a comparable reactivity score to the 18-month score, we also calculated a 36-month reactivity score of the mean of the four challenges minus the first resting condition. We used this second reactivity score for the 36-month-olds in post-hoc analyses comparing reactivity scores across the ages.

The RSA and PEP reactivity scores at 18- and 36-months were then converted into categorical variables: RSA reactivity score: 1 = RSA negative reactivity score (RSA reactivity < 0 or a negative number) and 0 = RSA positive reactivity score (RSA reactivity > 0 or a positive number) and PEP reactivity score: 1 = PEP negative reactivity score (PEP reactivity < 0 or a negative number) and 0 = PEP positive reactivity score (PEP reactivity > 0, or a positive number). There were some scores that came close to zero but none that were an absolute zero. These categorical RSA and PEP reactivity scores were then coded into profile scores at 18- and 36-months. A coactivation profile was coded as RSA reactivity = 0 and PEP reactivity = 1; coinhibition profile was coded as RSA reactivity = 1 and PEP reactivity = 0; reciprocal PNS activation/SNS not activated profile was coded as RSA reactivity = 0 and PEP reactivity = 0; and, reciprocal SNS activation/PNS withdrawal profile was coded as RSA reactivity = 1 and PEP reactivity = 1. If a participant did not have either an RSA or PEP reactivity score at 18- or 36-months of age, they were not assigned a profile.

Data Analysis

The statistical analysis program SPSS 25 was used to analyze and graph these data. Descriptive statistics were run for demographic characteristics, and descriptive statistics and histograms were run for RSA and PEP resting, challenge, reactivity, and ANS profile scores. Means, standard deviations, and Pearson correlations were used to understand the distribution of the continuous RSA and PEP reactivity data at 18- and 36-months. Cross-tabulations, Spearman correlations, and Chi-square statistics were run of the dichotomized RSA and PEP reactivity scores and of the ANS reactivity profiles. The cross-tabulation of the ANS profiles from 18- to

36-months revealed details of the within-individual stability of the ANS profiles. Due to these data being repeated measures and having cell sizes of less than five, the McNemar-Bowker test was used to calculate the stability of all four ANS profiles from 18- to 36-months of age.

Results

Children included in these analyses were 19.0 months old (*SD* = 1.3) on average, at the time of their 18-month-old visit (*n* = 134), and were 38.7 months (*SD* = 3.4) at the time of their 36-month-old visit (*n* = 102); 53% of participants at both visit points were girls. Based on the parent's classification of their child's race, the participants included in the 18-month-old visit analysis were 32% White, 14% were Black, and 54% Other (such as Asian, Native American, mixed race); 42% were classified as Hispanic. Among children included in 36-month-old visit analyses, 36% were White, 12% were Black, and 52% were Other, with 33% of the total further classified as Hispanic. The families' median annual household income was $19,000, ranging from $0 to $98,000. The mean household federal poverty level was less than 200% at the 18-month and 36-month-old visits.

The mothers' mean age at the time of delivery was 38 years (*SD* = 5.8) of age with a range from 18 to 43 years of age (*N* = 180). Sixty-eight percent of the mothers were married or had a partner and 54% were multiparous. Thirty-one percent of the mothers had a high school education or less, 50% had some college or vocational training, and 19% had earned a college degree. The families' median annual household income was $19,000, ranging from $0 to $98,000 with the majority of the sample falling 100% below the federal poverty level (FPL) at study enrollment. Eight-five percent of the mothers identified their ethnic or racial backgrounds as 39% African American, 31% Latina, 15% Caucasian, 2% Asian, and 13% other or multiracial.

The mother's cesarean rate was 28%, and infants' average gestational age at birth was 39.6 (*SD* = 1.4) weeks.

The frequency (n, %) of children categorized with a negative or positive RSA and PEP reactivity score at 18- and 36-months of age is shown in Table 3.2 as it is important to understand reactivity scores before they are converted into profiles. There were 91 (89%) children who had scoreable RSA reactivity data and 80 (78%) children who had PEP reactivity scores at both the 18- and 36-month timepoints. RSA reactivity was not significantly related from 18- to 36-months ($x^2 = 0.9(1)$, $p = 0.35$, $n = 91$) and PEP reactivity was not correlated from 18- to 36-months of age ($x^2 = 0.21(1)$, $p = 0.65$, $n = 80$).

To address the distribution of RSA and PEP in this cohort, the prevalence of children with RSA or PEP reactivity scores were compared from 18- to 36- months of age. There were 35% of children with a negative RSA reactivity score at 18-months of age compared to 82% of children with a negative RSA reactivity score at 36-months of age. There were 36% of children with a negative PEP reactivity score at 18-months of age compared to 59% of children with a negative PEP reactivity score at 36-months of age. For those with data at both timepoints, at 36-months, 31% of children had a negative RSA reactivity score; whereas, only 13% of children had a positive RSA reactivity score. For those with data at both timepoints, at 36-months, 23% of children had a negative PEP reactivity score and 28% of children had a positive PEP reactivity score.

The number of children who had ANS profile scores at both the 18- and 36-month timepoint ($n = 80$) are shown in Table 3.3. The cross-sectional data of the ANS profiles at 18- and 36-months of age reflected continuity compared to the repeated measures data. At 18 months, of all the children who fit an ANS profile ($n = 117$), 29 (25%) were in coactivation, 19

(16%) coinhibition, 54 (46%) reciprocal PNS activation/SNS not activated, and 15 (13%) reciprocal SNS activation/PNS withdrawal. At 36 months, of all the children who fit an ANS profile (n = 96), 10 (10%) were in coactivation, 33 (35%) coinhibition, 8 (8%) reciprocal PNS activation/SNS not activated, and 45 (47%) reciprocal SNS activation/PNS withdrawal. Of the four ANS profiles at 18-months, the reciprocal PNS activation/SNS not activated profile had the most children at 46%, but at 36-months, the reciprocal SNS activation/PNS withdrawal profile had the most children at 49%. There was a 39% decrease in the reciprocal PNS activation/SNS not activated profile, and a 34% increase in the reciprocal SNS activation/PNS withdrawal profile from 18- to 36-months of age.

The number of children with ANS profile data at both 18- and 36-months of age are shown in Table 3.4. Overall, there is a statistically significant difference in the ANS reactivity profiles from 18- to 36-months of age (McNemar = 37.72, $df = 6$, $p < .05$). The largest change of profiles from 18- to 36-months was from reciprocal PNS activation/SNS not activated to reciprocal SNS activation/PNS withdrawal (n = 18). Of the children at 18-months in the coactivation profile, 12% stayed in that profile at 36-months. Of the children at 18-months in the coinhibition profile, 43% stayed in that profile at 36-months. Of the children at 18-months in the reciprocal PNS activation/SNS not activated profile, 5% stayed in that profile at 36-months. Of the children at 18-months in the reciprocal SNS activation/PNS withdrawal profile at 36-months, 58% stayed in that profile at 36-months.

Discussion

The distribution of young children in three of the four ANS reactivity profiles significantly differed between 18- and 36-months of age and the proportion of children with the reciprocal SNS activation/PNS withdrawal profile was greater at 36-months than 18-months of age. The distribution of RSA and PEP reactivity from 18- to 36- months was different across the ages; however, there was high stability of RSA reactivity and moderate stability of PEP reactivity. There was a significantly different distribution of children among the ANS profiles from 18- to 36-months of age; although, there was some stability for one ANS profile, reciprocal PNS activation/SNS not activated. These results partially supported our hypotheses.

The strong stability of RSA reactivity and moderate stability of PEP reactivity from 18- to 36- months of age can be interpreted as a child who showed RSA reactivity at 18-months is likely to show RSA reactivity at 36-months. The same could be said for a child at 18-months who showed PEP reactivity will likely show PEP reactivity at 36-months. There is consistency of the ANS reactivity profiles at 18- and 36-months of age. By 36-months, most children were classified in the classic reactivity profile and the lowest prevalence was for children classified as the reciprocal PNS activation/SNS not activated profile. Figure 3.1 shows a summary of the research studies' frequency of ANS profiles and the most frequent profile for 36-month olds and older is the classic reactivity profile, as found in this study. There was a lot of movement in the prevalence of children characterized in each of the four profiles from one timepoint to the other. Our study also repeated a wide range of change from 5% to 58% of children having the same ANS reactivity profile at 18- and 36-months of age.

RSA resting measures in young children have been consistently shown to be moderately-stable (Calkins et al., 2008; El-Sheikh et al., 2009; Alkon et al., 2011) but children's RSA reactivity has not previously shown stability (Alkon et al., 2011). Of the 18-month-olds with a negative RSA reactivity score, the majority of them had a negative RSA reactivity score at 36-months of age which showed high stability of RSA reactivity from 18- to 36-months of age. Ellis, Essex, and Boyce in 2005 looked at PEP reactivity over a 7-year period and found that children who experience early adversities had a higher PEP reactivity at age 7 than younger ages. The moderate stability of PEP reactivity from 18- to 36-months found in this study detected stability of PEP reactivity younger than ever before. Early detection of PEP reactivity is important because one study found that mothers who experience high socioeconomic adversity during pregnancy have children who have less plasticity of their PEP reactivity from infancy to five years of age compared to children whose mothers experienced no adversities (Alkon et al., 2014). If a child exhibits a negative RSA or PEP reactivity score around toddlerhood, it is likely that the children will continue to be reactive through pre-school age. This time period can be crucial in which to employ interventions that address stress reduction for the child and the parent. There was a larger percent of children at 36-months who had a negative RSA and PEP reactivity score than at 18-months. Studies have shown that as the child gets older (up until 5-8 years old), there is an increase in the number of children with RSA and PEP reactivity (Alkon et al., 2003, Alkon et al., 2011). This may be due to children becoming more engaged in the challenges in the protocol than children at earlier ages.

At 18-months, the majority of children were characterized with reciprocal PNS activation/SNS not activated profile, but at 36-months, the majority of the children were characterized with the classic reactivity profile and the reciprocal PNS activation/SNS not activated profile was the least frequent. The CHAMACOS cohort study found similar results from 12- to 42-months of age. The CHAMACOS study had 34% of their 12-month-olds exhibiting the reciprocal PNS activation/SNS not activated, but at 42 months, 40% of the children exhibited classic reactivity (Alkon et al., 2011). These findings were stable from 42- to 60-months of age (Alkon et al., 2011; Alkon et al., 2017). The continuity of the shifts in profiles does infer that there may be a developmental pattern to stress reactivity. The continuity in profiles found in the CHAMACOS study is similar to the continuity in profiles found in this study. Children with reciprocal PNS activation/SNS not activated (low ANS reactivity) were more likely to have sleep problems (Alkon et al., 2017) but protected against delinquent behavior in parental relationships with marital problems (El-Sheikh et al., 2009). This is an example of where having low reactivity for one child could be a positive attribute whereas for another child it could be viewed as a negative attribute. Externalizing disorders have been associated with children who have the classic reactivity profile (Pearson et al., 2005). However, one of the earliest studies of profiles has acknowledged that the classic reactivity profile shows a normative response (SNS increase, PNS decrease) to stress (Solomon, Matthews, & Allen, 2000).

Children at 18-months did not keep their same ANS profile at 36-months of age; thus, found a lack of statistical evidence for the stability of the profiles. However, the greatest number of children stayed in the classic reactivity profile from 18- to 36-months of age which supports the finding of those with a negative RSA or PEP reactivity score are more likely to stay with that score through 36-months of age. The reciprocal PNS activation/SNS not activated profile did not have much representation at 36-months, but of those that did, 33% were that profile at 18-months. Our study did not come to any significant conclusions with the coactivation profile; however, the coinhibition profile had moderate stability and its representation almost doubled in number from 18- to 36-months of age. Not much is known about the coinhibition or coactivation profiles due to these profiles not being represented in a larger percent of the population. Coinhibition tends to be more prevalent the preschool years but over time becomes less prevalent (Quas et al., 2014, Alkon et al., 2011). Coinhibition has shown to be a moderator in the relationship of experiencing marital conflict and delinquency in children and having the coactivation profile has shown to be a protective factor (El-Sheikh et al., 2009).

Strengths and Limitations

This was the first study to evaluate ANS reactivity profiles at 18 months, and the longitudinal study design allowed the authors to characterize individual children's movement between profiles from 18- to 36-months of age. The significance of this contribution is apparent by the finding that this was a period of dramatic change in autonomic reactivity. Another strength is that we explicitly assessed how our findings compared with results from other studies collected at slightly younger and older age points, helping clarify the developmental trajectory of ANS profile distributions between six months and 7-8 years. Our study also contributed data from a racially and ethnically diverse sample, whereas previous research has come from either a low-income Mexican American sample (Alkon et al. 2011) or more affluent, predominantly Caucasian samples (Alkon et al. 2007). This study has a small sample size that could limit its statistical power for finding significant relationships. There were differences between the 18- and 36-month old protocols to accommodate for developmental changes; namely, the different challenge and resting tasks, the position of the child during the protocol, and the paired-control tasks during the 36-month old protocol. There could be concern for the lack of a standardized assessment of the home environment; however, about half of the assessments were completed in a standardized laboratory setting. Previous analyses found that when comparing the scores of the children's assessments completed in the home versus in the laboratory there was no significant difference between scores. Another limitation is the categorization of reactivity as above versus below zero instead of keeping reactivity as a continuous variable. Therefore, there may be misclassification in the PNS and SNS reactivity scores that can over- or under-estimate the findings.

Future directions

It is unclear why ANS profiles differ across research studies. Future studies should attempt to understand ANS profiles over time. Differences could be due to a child's age, the type of protocol, or certain characteristics such as poverty or adversity. Based on sample ANS means, it seems that the resting condition (lullaby) for our 18-month-old protocol was not as relaxing as the story read aloud at 36-months of age; thus, future studies should pilot test alternative resting conditions for toddlers to engage them while eliciting a calm state. The use of two separate categorization systems - Bernston et al. (1991) ANS reactivity profiles versus the ACM-guided patterns – made it difficult to interpret the results of these studies relative to one another. Assessing both categorization systems in parallel within several study samples could help clarify how the two systems relate to one another empirically (e.g., to quantify the degree of overlap between theoretically-similar "coinhibition" profile and "buffered" pattern). It would also be useful to evaluate whether one system's categorizations are more strongly associated with outcomes of clinical interest (e.g. externalizing problems), so as to assess which system holds more promise to guide possible clinical interventions.

Conclusion

This analysis of ANS reactivity profiles between 18- and 36-months of age has helped fill in an age gap in the growing literature regarding developmental patterns of ANS reactivity. While previous literature suggested that a major shift in profile distributions occurred some point between 12 and 42 months (Alkon et al. 2011), this analysis narrows that age band and suggests that the shift occurs between 18 and 36 months. As the field of ANS research progresses toward clinical applications, this finding may inform optimal timing of interventions intended to lessen the effects of stressful experiences that occur during sensitive periods. These findings may support the assessment of ANS reactivity in the clinical setting which can be applied to decision making for various mental and physical illnesses.

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Table 3.1 Autonomic Nervous System Profiles in Relation to Autonomic Nervous System Activity, Respiratory Sinus Arrhythmia and Preejection Period Reactivity, and Adaptive Calibration Model Patterns

ANS[1] Profile	PNS[2] Activity	SNS[3] Activity	RSA[4] Reactivity[6,7]	PEP[5] Reactivity[6,7]	ACM[8] Pattern
Coactivation	activated	activated	+	-	Sensitive
Coinhibition	inhibited	inhibited	-	+	Buffered
Reciprocal PNS activation/SNS not activated (aka low reactivity)	activated	inhibited	+	+	Unemotional
Reciprocal SNS activation/PNS withdrawal (aka classic reactivity)	inhibited	activated	-	-	Vigilant

[1]ANS: autonomic nervous system; [2]PNS: parasympathetic nervous system; [3]SNS: sympathetic nervous system; [4]RSA: respiratory sinus arrhythmia; [5]PEP: pre-ejection period; [6] +: positive reactivity score; [7] -: negative reactivity score; [8]ACM: Adaptive Calibration Model

(Alkon et al., 2002, 2011, 2017; Berntson et al., 1994, 1996; Berntson, Cacioppo, & Quigley, 1991, 1993; Cacioppo et al., 1994; Salomon, Matthews, & Allen, 2000)

Table 3.2 Frequencies of Respiratory Sinus Arrhythmia (RSA) and Preejection Period (PEP) Reactivity at 18- and 36-Months of Age

	18 months		**36 months**	
	RSA (n %)	**PEP** (n %)	**RSA** (n %)	**PEP** (n %)
Negative Reactivity Score	32 (35%)	29 (36%)	75 (82%)	47 (59%)
Positive Reactivity Score	59 (65%)	51 (64%)	16 (18%)	33 (41%)
Total	91 (100%)	80 (100%)	91 (100%)	80 (100%)

Table 3.3 Frequencies of Autonomic Nervous System (ANS) Reactivity Profiles for Children Who Participated at Both 18 and 36 Months of Age

ANS Profile	***18 months***	***36 months***
Coactivation of SNS[1] and PNS[2]	17 (21%)	8 (10%)
Coinhibition of SNS and PNS	14 (18%)	27 (34%) *
Reciprocal PNS activation/SNS not activated	37 (46%)	6 (7%) *
Reciprocal SNS activation/PNS withdrawal	12 (15%)	39 (49%) *
Total	**80 (100%)**	**80 (100%)**

[1]SNS: sympathetic nervous system, [2]PNS: parasympathetic nervous system
*McNemar = $p < .05$

Table 3.4 The Number of Children with Autonomic Nervous System (ANS) Reactivity Profiles at both 18- and 36-Months of Age and % of 18-Month-Olds with the Same Profile at 36-Months of Age

		36 months				
	ANS Profile	**Coactivation**	**Coinhibition**	**Recip PNS***	**Recip SNS****	**Total**
18 months	**Coactivation**	2 (12%)	6	2	7	17
	Coinhibition	0	6 (43%)	1	7	14
	Recip PNS*	4	13	2 (5%)	18	37
	Recip SNS**	2	2	1	7 (58%)	12
	Total	8	27	6	39	80

McNemar = 37.72 (df = 6), p <.05, n = 80
*Recip PNS: reciprocal parasympathetic activation/sympathetic not activated
**Recip SNS: reciprocal sympathetic activation/parasympathetic withdrawal

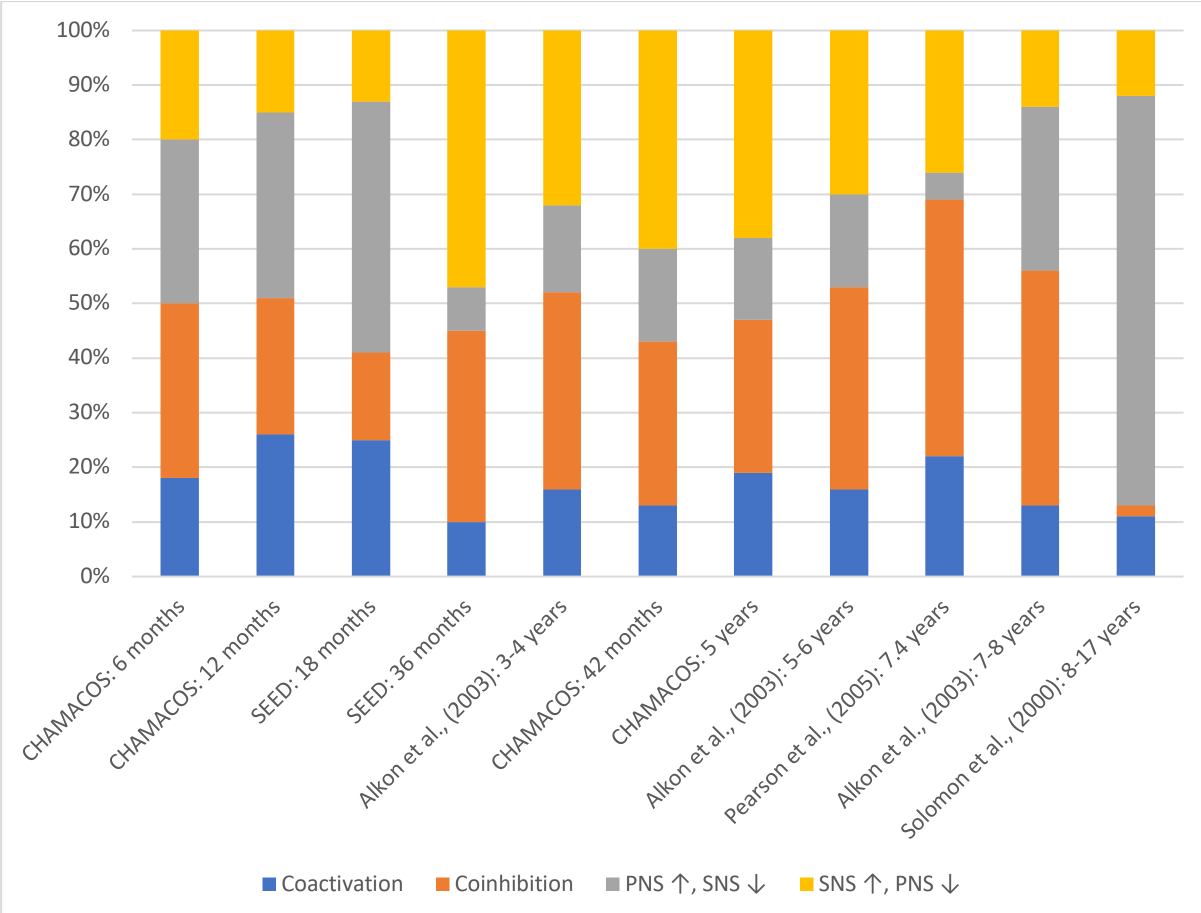

Figure 3.1 Frequency of ANS Profiles by Study Grouped by Ages
CHAMACOS: The Center for the Health Assessment of Mothers and Children of Salinas Study
SEED: Stress, Eating, and Early Development Study
PNS: parasympathetic nervous system
SNS: sympathetic nervous system

Chapter Four: Third Manuscript

The Sociodemographic Context of
Autonomic Nervous System Reactivity in Early Childhood

Abstract

Background: Sociodemographic characteristics can provide knowledge of a child's environment, or the surroundings that provide context for human engagement. Cardiac autonomic nervous system (ANS) measurements, such as respiratory sinus arrhythmia (RSA) and pre-ejection period (PEP), have been shown to be valid and reliable indicators of children's sensitivity to stressors in their environment. However, little is known about how a child's sociodemographic characteristics may influence ANS reactivity. Limited data has shown living in poverty is related to greater PEP reactivity in children; however, other demographics such as biological sex, race, and ethnicity have an unclear relationship with ANS reactivity. The purpose of this study is to identify the relationship between selected sociodemographic characteristics (children's biological sex, race, ethnicity, and federal poverty level (FPL)) and children's multisystem ANS reactivity profiles within a sample of low-income families, at 18- and 36-months of age.

Methods: At 18-months (n = 134) and 36-months (n = 102) of age, children completed a developmentally challenging protocol that simultaneously assessed their RSA and PEP under resting and challenge conditions. Four ANS profiles (coactivation, coinhibition, reciprocal parasympathetic (PNS) activation/sympathetic (SNS) not activated, and reciprocal SNS activation/PNS withdrawal) were created by dichotomizing reactivity, mean challenge minus rest, as positive or negative reactivity, and combining RSA and PEP reactivity scores.

Results: About half of the study participants were girls, approximately 40% Hispanic, mostly mixed race, and about 70% lived in households < 200% of the federal poverty level. Race (x^2= 15.3(*6*), p = 0.02, n = 117) and FPL (x^2 = 12.5(*3*), p < 0.01, n = 117) were significantly different across the four ANS profiles at 18-months of age. At 18-months, logistic regression models revealed that children living < 200% of the FPL had significantly higher odds of the coactivation profile (*OR* = 3.1(*1*), p = 0.02, *CI* = 1.2-7.6) and lower odds of the coinhibition profile (*OR* = 0.1(*1*), p = 0.02, *CI* = 0.01-0.69) compared to children living > 200% of the FPL. At 36-months, Hispanic children had higher odds of the reciprocal SNS activation/PNS withdrawal profile (*OR* = 2.7(*1*), p < 0.05, *CI* = 1.00-7.25) compared children who were not Hispanic.

Conclusion: These findings show that between toddlerhood and preschool age, most of the sociodemographic characteristics are not associated with a child's ANS reactivity profile. Although most of these demographic characteristics do not predict ANS reactivity they may modify or mediate the relations between environmental stress and ANS reactivity.

Keywords: autonomic nervous system, respiratory sinus arrhythmia, preejection period, sympathetic nervous system, parasympathetic nervous system, early childhood, psychobiological, sociodemographics

Introduction

There is evidence that children who are male (Solomon, Matthews, & Allen, 2000), African American (Wagner et al., 2016), or living in poverty (Alkon et al., 2011; Ellis, Essex, & Boyce, 2005) have greater PEP reactivity, indicating an increased "fight or flight" impulse during a challenged state compared to a resting state. Therefore, the relationship between inter-individual contexts and young children's autonomic nervous system (ANS) responsivity is an important intersection to study in the field of psychophysiological research. According to the Gene x Environment Interaction model, physical, chemical, and built environmental factors can affect adult health by becoming biologically embedded during sensitive developmental periods (Hertzman, 2013b), such as in early childhood, and/or accumulate damage over time (Johnson, 2005; Shonkoff, 2010). Hertzman's theory of biological embedding explains that if children grow up in healthy environments, they would be less likely to have early disease onset and the society would have smaller disparities in health status and income (Hertzman & Boyce, 2010). Environmental influences are often underreported in psychophysiological research (Gatzke-Kopp, 2016). Constructs such as biological sex, race, ethnicity, and socioeconomic status may play a role in understanding the process by which the autonomic nervous system responds and develops over time.

The ANS has two main branches, the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). Each ANS branch has a cardiac measurement; respiratory sinus arrhythmia (RSA) reflects PNS activity, and preejection period (PEP) reflects SNS activity. RSA and PEP have been shown to be valid and reliable indicators of children's sensitivity to changes in their environment (Bernston et al., 2007; Alkon et al., 2003). The categorization of RSA and PEP reactivity scores into ANS reactivity profiles, specifically coactivation (a positive

RSA reactivity score and a negative PEP reactivity score), coinhibition (a negative RSA reactivity score and a positive PEP reactivity score), reciprocal parasympathetic (PNS) activation/sympathetic (SNS) not activated (a positive RSA reactivity score and a positive PEP reactivity score), and reciprocal SNS activation/PNS withdrawal (a negative RSA reactivity score and a negative PEP reactivity score), elucidate two-dimensional patterns of ANS responsivity (Berntson et al., 1991). If the ANS becomes chronically dysregulated or continually differs from normative ANS patterns in the general population (Alkon et al., 2012), physical and mental health problems may occur (Bauer et al., 2002; McEwen, 1998).

Cardiac autonomic nervous system (ANS) measurements, such as respiratory sinus arrhythmia (RSA) and pre-ejection period (PEP), have been shown to be valid and reliable indicators of children's sensitivity to stressors in their environment; however, there is little knowledge of these indices categorized by ANS profiles and examined in relationship to common sociodemographic characteristics in young children.

Biological Sex and ANS Reactivity

Notably, a meta-analysis of ANS functioning during the Still-Face Paradigm (SFP), which is conducted during the first year of life, has found only four of 33 studies do show differences of ANS resting by child's sex within the first year of life (Jones-Mason et al., 2018), with patterns of significant findings inconsistent. Of these studies, two have shown that girls have lower resting RSA compared to boys (El-Sheikh, 2005; van Dijk et al., 2012) while two showed that girls have higher resting RSA than boys (Fabes et al., 1994; Gordis et al., 2010).

Generally, studies that report associations between a child's biological sex and ANS reactivity appear to follow a pattern congruent to when a child's sex most affects their physical and/or mental development. Studies have shown biological sex-based differences in ANS reactivity within the first year of life (Tibu et al., 2014; Moore et al., 2009). During the early and middle childhood years, evidence suggests that RSA reactivity does not differ by sex (Alkon et al., 2003), but PEP reactivity may differ by sex (Bush et al., 2017; Salomon, Matthews, & Allen, 2002). Sex differences have been found around the time of puberty (Salomon et al., 2002).

A study evaluated the stability of the stress response over a three-year period (Matthews et al., 2002). There were 100 children enrolled at eight to ten years of age and examined again at 15 to 17 years of age (n = 49), of which, 24% were Black female and 25% were White female, and 22% were Black male and 29% were White male. Systolic (SBP) and diastolic blood pressure (DBP), and PEP were collected while administering a three-task protocol (reaction time, mirror tracing, and cold forehead) with resting tasks before and after the challenge tasks. Males displayed higher resting SBP compared to females (F (1,136) = 29.6, $p < 0.001$). Also, males had a greater PEP reactivity compared to females from 15-17 years of age (Matthews et al., 2002).

A cross-sectional study of 273 three to eight-year old children of mostly White, college graduate mothers with a family income of $60,000 or above, examined ANS reactivity profiles in relation to demographic characteristics in three different studies and settings (e.g., child's home, child care center, or research laboratory) (Alkon et al., 2003). A developmentally challenging protocol was administered that included a 2-minute relaxing story read aloud at the beginning and end of the protocol as the resting condition and the challenge tasks elicited four different domains (social, cognitive, physical, and emotional). The children's RSA and PEP reactivity scores were dichotomized as positive or negative and categorized into the four profiles (coactivation, coinhibition, reciprocal PNS activation/SNS not activated, and reciprocal SNS activation/PNS withdrawal) proposed by Berntson et al. in 1991. They found that ANS profiles did not differ by sex from three to eight years-old.

The confounding effects of psychomotor activity on ANS reactivity (RSA and PEP) was assessed in a study of 338 five- and six-year-old children (Bush et al., 2017). The study included a standardized, developmentally challenging protocol (Alkon et al., 2003; Boyce et al., 1995) with four domains of challenge tasks (social, cognitive, sensory, and emotional), each preceded by a task-specific resting condition in an attempt to narrow the degree of psychomotor activity. Notably, few differences in ANS measures by child's sex were found. Psychomotor activity levels were not different by child's sex for either RSA or PEP resting. Challenge reactivity (mean challenge task minus the mean non-challenging comparison task) and overall task response (mean challenge task minus the mean resting task) were not different by child's sex for RSA reactivity. PEP reactivity was statistically significantly greater for boys than for girls (F $(1, 315) = 6.80$, $p < 0.01$). PEP cognitive overall task response (F $(1, 317) = 3.90$, $p < 0.05$) and sensory overall task response (F $(1, 310) = 5.12$, $p = 0.02$) were statistically significantly greater for girls than boys. All other PEP summary scores were not different by child's sex. Collectively, only three of the 26 ANS scores showed child's sex differences, suggesting that child's sex differences were not a major factor for 5- and 6-year-olds who completed a developmentally challenging protocol (Bush et al., 2017).

Overall, there is no conclusive evidence of RSA reactivity differences by children's biological sex; however, there is some evidence that boys may have greater PEP reactivity compared to girls.

Race and Ethnicity and ANS Reactivity

Racial or ethnic differences by ANS reactivity has not been examined in many studies. Race is a social construct and, thus, is not attributed to biologic characteristics (Smedley & Smedley, 2005). If the understanding of racial and ethnic differences by ANS reactivity is approached or treated insensitively, there can be detrimental offenses such as misuse of data that can harm populations in discriminatory ways, which make exploring such constructs difficult yet important.

A few studies (Holochwost et al., 2014; Moore et al., 2009; Quigley et al., 2016) included in a meta-analysis of ANS responsivity and the still-face paradigm in infants found a statistically significant difference based on race, with African American children having a higher resting RSA compared to European American children (Jones-Mason et al., 2018).

Murphy et al. (1995) were the first researchers to explore the differences in cardiovascular reactivity and development from adolescence to adulthood between, Black and White children with a mean age of 9.1 years in Memphis, Tennessee. Under stress, Black children had significantly higher systolic and diastolic blood pressure and heart rates compared to White children. Allen & Matthews (1997) echoed these results when evaluating Black and White children as they transitioned from middle childhood to adolescence in Pennsylvania.

Salomon, Matthews, & Allen in 2000 explored patterns of PNS and SNS reactivity in children and adolescents. Of the 201 participants ages eight to ten years of age and 15-17 years of age at time of recruitment, they were split nearly evenly between Black (48%) and White (51%) participants. They collected heart rate (HR), PEP, and other cardiovascular reactivity measurements. Whites showed greater HR variability and exhibiting a greater proportion of parasympathetic activation relative to Black participants ($F = (1, 175) = 4.42$, $p = 0.036$) only during the challenge episodes (Salomon, Matthews, & Allen, 2000). This finding suggests that Black participants did not show the buffering effect of parasympathetic activation on HR reactivity.

The relationship between ethnicity and ANS reactivity was explored in a longitudinal cohort study among young Latino children in the face of prenatal adversity (Alkon et al., 2011). The sample of 378 children, 62% living at or below the federal poverty level, of young Latinx mothers (< 30 years of age), were assessed at 6-, 12-, 42-, and 60- months of age. A developmental challenges protocol was administered primarily in Spanish that included a 2-minute relaxing story read aloud to the 42- and 60- month olds or a lullaby played on a laptop for the 6- and 12-month olds at the beginning and end of the protocol as the resting condition and the challenge tasks elicited across four different domains of development (social, cognitive, physical, and emotional). The study found that for mothers who had at least one social support, their children who experienced a single adversity had less ANS reactivity than for those who experienced zero or two adversities. As there was no comparison group, we can only interpret the findings within a homogenous sample of low-income Latino families.

Wagner and colleagues in 2016 examined the relationship between RSA resting and race from a prospective longitudinal study of 206 children at 6-, 12- and 36-months of age who were 56% African-American, 44% European-American, and 53% below 200% of the federal poverty level. The protocol consisted of mother-child free play task structured for 10 minutes. They did not find a relationship between race and RSA resting at any timepoint. Overall, these studies found no conclusive evidence of a relationship between race and ethnicity and ANS reactivity; however, some evidence shows a relationship between being Black and having higher RSA or PEP reactivity (Wagner et al., 2016).

Poverty and ANS Reactivity

Adverse early childhood experiences can arise from living in poverty and, in turn, affect an individual's mental and physical health (Felitti et al., 1998). There are several studies that show that there is a relationship between children who live in poverty and their ANS reactivity. Alkon et al. (2012) elucidated the relationship between poverty and its effects on the ANS summarizing the associations between poverty and ANS dysregulation. ANS dysregulation occurs when the ANS does not calm down after a stressful experience or the ANS does not turn on when a stressful experience occurs (Bauer, Quas, & Boyce, 2002).

A couple of studies illuminate the concept of ANS dysregulation in relationship to the experience of living in poverty. A study by Evans & Kim in 2007 has shown that the ANS may not be as responsive when chronic, stressful experiences occur. They have demonstrated that adolescents who have lived in chronic poverty since birth have lower blood pressure reactivity compared to adolescents who have lived fewer years in poverty (Evans & Kim, 2007). A study by Ellis, Essex, & Boyce in 2005 has shown how the ANS does not calm down after experiencing stress. This was a cross-sectional study of school-age children (mean age = 6.9 years) who experienced multiple life stressors (including financial stress and low socioeconomic status) in the first five years of life. These children had higher sympathetic reactivity than children who experienced fewer life stressors (Ellis, Essex, & Boyce, 2005).

El-Sheikh et al. (2009) studied how a child living in poverty is susceptible to externalizing problems associated with marital conflict by studying the ANS responsivity. The study included 176 children (56% girls) attending the third grade at a public school in the southeastern USA and their parents who were of low socio-economic status with a median family income $35,000 to $50,000 (El-Sheikh et al., 2009). RSA and skin conductance reactivity measures were collected. They found that the ANS profiles of coactivation and coinhibition exacerbated the association between interparental conflict and children's externalizing symptoms.

Conversely, the reciprocal PNS activation/SNS not activated profile buffered the association between conflict and symptoms (El-Sheikh et al., 2009).

Alkon et al. (2011) described ANS reactivity of children living in poverty over their first five years of life. Using a standardized protocol, RSA and PEP were measured in a sample of participants who took part in a larger, longitudinal birth cohort study called the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS). This study had 378 mostly Latino participants at six, 12, 42, and 60 months of age. Reactivity profiles showed developmental changes such as decreased heart rate, increased RSA, and increased PEP. The most significant change was an increase in the reciprocal SNS activation/PNS withdrawal (classic reactivity) profile that increased from 19.5% to 37.0% as children developed from 6 months old to 60 months old. The percent of children in the classic reactivity profile increased by 27% with every year of life. These findings are important because previous studies have shown that children with the classic reactivity profile are at high risk of having externalizing problems if they live in conditions of poverty or adversity (El-Sheikh et al., 2009; Pearson et al., 2005).

Alkon et al. (2014) studied whether maternal exposure to psychosocial risk factors during pregnancy, such as poverty or low social support, predicted trajectories of ANS reactivity measures in mothers' offspring from six months and five years of age. No associations were found between prenatal adversity and the child's ANS reactivity at specific ages (six months, one year, 3.5 years, and five years of age). However, low socioeconomic status or low social support predicted lower heart rate (HR) and less sympathetic nervous system (SNS) reactivity (but not parasympathetic nervous system reactivity) trajectories from six months to five years of age compared to those children who had mothers who experienced no socioeconomic adversity or issues with social support. This study's outcome is different than the majority of studies that have found that young children living in poverty have greater ANS reactivity or a classic reactivity profile.

Operational Definitions

Child's *sex* is defined in this study as the child's biological sex, either female or male. Child's *race* is defined in this study as maternal report of whether the child is Caucasian, African American or Other classification (Asian, Native American, or mixed race) (Centers for Disease Control and Prevention, 2015). Collapsing categories enhanced power for statistical analysis. Child's *ethnicity* is defined in this study as either Hispanic or non-Hispanic. *Poverty* is defined in this study as < 200% below the federal poverty level (FPL) which is the household's FPL in which the child lives (United States Census Bureau, 2018). This measurement represents the percent below the designated FPL for the year in which the child's household income information was collected. This was is a categorical variable with two categories: < 200% of the FPL or > 200% of the FPL. The *coactivation* profile is defined by a positive RSA reactivity score and a negative PEP reactivity score (Berntson et al., 1991). The *coinhibition* profile is defined by a negative RSA reactivity score and a positive PEP reactivity score. The *reciprocal parasympathetic (PNS) activation/sympathetic (SNS) not activated* profile is defined by a positive RSA reactivity score and a positive PEP reactivity score. The *reciprocal SNS activation/PNS withdrawal (aka classic reactivity)* is defined by a negative RSA reactivity score and a negative PEP reactivity score.

Aim and Hypotheses

The purpose of this study is **t**o identify the relationship between selected sociodemographic characteristics (children's biological sex, race, ethnicity, and federal poverty level (FPL)) and children's ANS reactivity profiles (coactivation, coinhibition, reciprocal PNS activation/SNS not activated, and reciprocal SNS activation/PNS withdrawal) at 18- and 36-months of age. The hypotheses are: a) the overall model of child's sex, race, ethnicity, and FPL will significantly predict RSA and PEP reactivity at 18- and 36-months of age, b) child's sex, race, ethnicity, and FPL will not significantly predict the coactivation, coinhibition, or reciprocal PNS activation/SNS not activated profile but will significantly predict the reciprocal SNS activation/PNS withdrawal profile at 18- and 36-months, c) child's sex will not be a significant independent predictor of any of the four ANS reactivity profiles, controlling for FPL, race, and ethnicity at 18- and 36-months, d) child's race will predict the classic ANS reactivity profile, controlling for child's sex, ethnicity, and FPL at 18- and 36-months, e) child's ethnicity will predict the classic ANS reactivity profile, controlling for child's sex, race, and FPL at 18- and 36-months, and f) child's FPL will predict the classic ANS reactivity profile, controlling for child's sex, race, and ethnicity at 18- and 36-months.

Methods

This cohort study is part of a larger birth cohort study, the Stress, Eating, and Early Development (SEED) study, which was a continuation of the Maternal Adiposity, Metabolism, And Stress (MAMAS) study. The MAMAS study was a non-randomized control trial designed to examine the effects of a mindfulness-based stress reduction and healthy lifestyle intervention to reduce excessive gestational weight gain among pregnant women (Bush et al., 2017). The SEED study followed the children of MAMAS mothers from birth through age five, investigating the impact of prenatal and early childhood stress and eating habits on the children's behavioral, physiologic, and anthropometric development. This paper describes and analyzes the sample and the ANS data collected at the 18-month and 36-month visits.

Participants in the MAMAS study were pregnant women recruited between August 2011 and June 2013 when they were between their 16th and 22nd week of pregnancy. Women were eligible to participate if they were of low to middle income and overweight or above. Recruitment took place at hospital-based clinics; community health centers; Supplemental Nutrition Program for Women, Infants and Children (WIC) offices; organizations providing services to pregnant women; and through online advertisements (e.g., Craigslist). In total, 220 women enrolled in the MAMAS study, of whom 215 remained involved in the study at delivery of a liveborn child. All of the 215 mothers were invited to enroll themselves and their newborns in the follow-up SEED study at delivery. In total, 180 mother-child dyads (84%) enrolled in SEED, of whom 134 children (74% of the enrolled delivery sample) and 102 children (57% of the delivery sample) participated fully at 18-month and 36-month study visits, respectively.

Data Collection

For both the 18- and 36-month visits, a trained research assistant met with the mother and child in the family's home or research laboratory and the dyad were together during the study visit. During these visits, the research assistant administered questionnaires with the mother, took anthropometric measurements of the child, and conducted a standardized, age-specific Developmental Challenges Protocol (DCP) with the child while simultaneously monitoring their ANS activity (RSA and PEP). The sociodemographic characteristics included in this paper, child's sex, race, ethnicity, and federal poverty level (FPL) were ascertained from these questionnaires.

Instrumentation

The self-reporting questionnaires used asked mothers about their demographics. The MAMAS infant database contained information (such as child's biological sex and age) from the child's medical record.

ANS Measures

The research assistant placed four spot electrodes on the child's neck and trunk to collect impedance and respiratory measures and three spot electrodes were placed on the right clavicle, lower left rib, and right abdomen for ECG measures (Bush et al., 2016). The ECG and impedance waveforms collected during the DCP yielded measures of HR, RSA, PEP, and respiratory rate (RR). Data were acquired using Mindware Technologies, LTD hardware (www.mindwaretech.com); continuous ECG, Zo (basal impedance), and d*Z*/d*t* (first derivative of the impedance signal) waveforms were recorded. A 4-milliamp AC current at 100 Hz was passed through the two current electrodes; Zo and dZ/d*t* signals were acquired from the two voltage recording electrodes.

The DCP was designed to elicit ANS responses to developmentally-appropriate challenges across different domains: cognitive, physical, and socioemotional and a comparison resting state (Table 2.1). After the electrodes were in place for five minutes, the DCP was started. The DCPs include age-appropriate resting activities with 18-month olds listening to a lullaby and 36-month olds listening to a story read aloud. The challenges varied at each age to be developmentally appropriate and at 36-months the protocol included task-specific challenges since the children were able to stay engaged for a longer period of time compared to the 18-month olds. These protocols were adapted from existing standardized protocols (Alkon et al., 2003, 2006, Bush et al., 2011) and pilot tested to assess the modified protocols were engaging, developmentally challenging, and tolerable for each age group.

All study protocols and consent forms were approved by the Institutional Review Board of the University of California, San Francisco.

Data Preparation and Scoring

Autonomic nervous system data were filtered, extracted, and then scored using Mindware Technologies, LTD software programs (HRV 3.1.0F and IMP 3.1.0H). RSA indices are calculated using the interbeat intervals on the ECG waveform, respiratory rates derived from the impedance waveform (e.g., d*Z*/d*t* signal), and a bandwidth range setting of 0.15 to 1.04 (Zisner & Beauchaine, 2015). PEP is measured in milliseconds as the time interval between the onset of ventricular depolarization (Q point on the ECG wave) and the onset of left ventricular ejection (B point on the d*Z*/d*t* wave).

Each 30 second interval or epoch was scored if there was a minimum of 20 seconds of clean data. (Zisner & Beauchaine, 2016). The task scores were calculated as the mean of several 30-second epochs depending on the task length (Table 2.1). Outliers were defined as RSA or PEP scores greater than three standard deviations from the sample mean for each 30-second epoch. For the 18-month-olds, there were seven outliers at the epoch level and five outliers at the overall task level. For the 36-month-olds, there were 16 outliers at the epoch level and three at the overall task level. After reviewing the raw data of these outliers with an ANS expert on our team, we decided to keep most of them in the analyses because their other scores showed similar results and were consistent with their individual patterns of physiology. We deleted 12 outliers for 36-month-olds at the epoch level that differed from their other raw data.

For the 18-month data, RSA and PEP reactivity scores were calculated as the mean response across the three challenge tasks minus the mean of the first resting condition. Reactivity scores were calculated for children who had ANS scored data for at least two of the three challenge tasks. As the 36-month-old protocol included task-specific matched control conditions, 36-month-old RSA and PEP reactivity scores were calculated as the mean of the three difference scores. Each difference score was calculated as the challenge minus the corresponding matched control condition response. The receptive vocabulary challenge was not included in these analyses because there was no task-specific matched control condition. Reactivity scores were only calculated for children with scorable data for at least two of the three different challenge/control difference scores. These reactivity scores were used in the analyses for this paper. Therefore, the reactivity scores for the 18-month-old and 36-month-old protocol were calculated differently because of the inclusion of task-specific control conditions in the 36-month-old DCP (Table 2.1). To calculate a comparable reactivity score to the 18-month score, we also calculated a 36-month reactivity score of the mean of the four challenges minus the first resting condition. We used this second reactivity score for the 36-month-olds in post-hoc analyses comparing reactivity scores across the ages.

The RSA and PEP reactivity scores at 18- and 36-months were then converted into categorical variables: RSA reactivity score: 1 = RSA negative reactivity score (RSA reactivity < 0 or a negative number) and 0 = RSA positive reactivity score (RSA reactivity > 0 or a positive number) and PEP reactivity score: 1 = PEP negative reactivity score (PEP reactivity < 0 or a negative number) and 0 = PEP positive reactivity score (PEP reactivity > 0, or a positive number). There were some scores that came close to zero but none that were an absolute zero. These categorical RSA and PEP reactivity scores were then coded into profile scores at 18- and

36-months. A coactivation profile was coded as RSA reactivity = 0 and PEP reactivity = 1, coinhibition profile was coded as RSA reactivity = 1 and PEP reactivity = 0, reciprocal PNS activation/SNS not activated profile was coded as RSA reactivity = 0 and PEP reactivity = 0, and reciprocal SNS activation/PNS withdrawal was coded as RSA reactivity = 1 and PEP reactivity = 1. If a participant was missing either an RSA or PEP reactivity score at 18- or 36-months of age, a profile could not be assigned.

Data Analysis

The statistical analysis program SPSS 25 was used to analyze and graph these data. Descriptive statistics were calculated for sociodemographic characteristics, and descriptive statistics and histograms were computed for RSA and PEP resting, challenge, reactivity, and ANS profile scores. Spearman correlations were calculated for all sociodemographic variables, RSA and PEP reactivity scores, and ANS profiles at both timepoints. Crosstabulations and Chi-square statistics were calculated between the sociodemographic characteristics and the dichotomized RSA and PEP reactivity scores and of the ANS reactivity profiles at both timepoints. Furthermore, the relationships between sociodemographic characteristics and RSA and PEP reactivity as well as sociodemographic characteristics and ANS profiles at 18- and 36-months, with predictors all entered simultaneously, were modeled using logistic regression due to having dichotomous, dependent variables.

Results

The mothers' mean age at the time of delivery was 38 years (SD = 5.8) of age with a range from 18 to 43 years of age (N = 180). Sixty-eight percent of the mothers were married or had a partner and 54% were multiparous. Thirty-one percent of the mothers had a high school education or less, 50% had some college or vocational training, and 19% had earned a college degree. The families' median annual household income was \$19,000, ranging from \$0 to \$98,000 with the majority of the sample falling 100% below the federal poverty level (FPL) at study enrollment. Eight-five percent of the mothers identified their ethnic or racial backgrounds as 39% African American, 31% Latina, 15% Caucasian, 2% Asian, and 13% other or multiracial. The mother's cesarean rate was 28%, and infants' average gestational age at birth was 39.6 (SD = 1.4) weeks. The mean age of the children included in these analyses at the 18-month visit was 19.0 months old (SD = 1.3) (n = 134) and at the 36-month visit they were 38.7 months (SD = 3.4) (n = 102).

Tables 4.1 and 4.2 present the sociodemographic characteristics according to autonomic nervous system profile by age. Of the 117 children who had ANS profile scores at 18-months, 52% were girls, 44% were Hispanic, 32% were Caucasian, 14% were African American, and 54% were Other (Asian, Native American, or mixed race), and 67% lived in families earning less than 200% FPL (see Table 4.1). Of the 96 children who had ANS profile scores at 36-months, 53% were girls, 34% were Hispanic, 37% were Caucasian, 11% were African American, and 52% were Other (Asian, Native American, or mixed race), and 75% lived in families earning less than 200% FPL (see Table 4.2).

Race and ethnicity were significantly associated with one another and so, to avoid collinearity, we included only ethnicity in the models.

The 18-month-old children who lived in families who were of lower income (< 200% of the FPL) were 3.2 times the odds of having a negative PEP reactivity score compared to the 18-month-old children who lived in families who were of higher income (OR = 3.2(*1*), $p < 0.01$, CI: 1.4-7.4) (see Table 4.3). There were no significant relations between children's sociodemographic characteristics and their 18-month-old RSA reactivity and 36-month-old RSA and PEP reactivity.

The most common profile for 18-month-olds for each selected sociodemographic (child's sex, race, ethnicity, and FPL) was the reciprocal PNS activation/SNS not activated profile (45-47%) (see Table 4.1). Child's sex was associated with the coactivation profile at 18-months (x^2 = 4.8(*1*), *p* = 0.03, *n* = 117). Boys were more likely to have this profile (*n* = 19 (16%)) compared to girls (*n* = 10 (8%)). Overall, child's race was associated with ANS profiles at 18-months of age (x^2= 15.3(*6*), *p* = 0.02, *n* = 117). Child's race was associated with the coactivation profile at 18-months (x^2 = 14.1(*2*), *p* < 0.01). Those classified as Other (*n* = 12 (10%)) were more likely than Caucasian (*n* = 7 (6%)) or African American (*n* = 10 (9%)) to have the coactivation profile. Overall, being < 200% of the FPL was associated with ANS profiles at 18-months of age (x^2 = 12.5(*3*), *p* < 0.01). Child's FPL was associated with the coactivation profile at 18-months (x^2 = 7.6(*1*), *p* < 0.01). Those living > 200% of the FPL were more likely (*n* = 15 (14%)) to have the coactivation profile compared to those living < 200% of the FPL (*n* = 13 (12%)). Child's FPL was associated with the coinhibition profile at 18-months (x^2 = 7.1(*1*), *p* < 0.01). Those living < 200% of the FPL are more likely to have the coinhibition profile (*n* = 17 (15%) compared to those living > 200% of the FPL (*n* = 1 (1%)).

The overall model was statistically significant when evaluating the relationship between the coactivation profile and the selected sociodemographic characteristics (child's sex, race, ethnicity, and FPL) at 18-months of age (x^2 = 10.9(*3*), *p* < 0.01, *n* = 117). The 18-month-old children who lived in families who were of lower income (< 200% of the FPL) were 3.1 times the odds of having a coactivation profile compared to the 18-month-old children who lived in families who were of higher income (> 200% of the FPL) (*OR* = 3.1(*1*), *p* = 0.02, *CI* = 1.2-7.6).

The overall model was statistically significant when evaluating the relationship between the coinhibition profile and the selected sociodemographic characteristics at 18-months of age ($x^2 = 10.4(3)$, $p < 0.01$). The 18-month-old children who lived in families who were of lower income (< 200% of the FPL) were 10 times lower odds of having a coinhibition profile compared to the 18-month-old children who lived in families who were of higher income (> 200% of the FPL) (*OR* = 0.1(*1*), $p = 0.02$, *CI* = 0.01-0.69) (see Table 4.3).

The most common profile for 36-month-olds for each selected sociodemographic (child's sex, race, ethnicity, and FPL) was the reciprocal SNS activation/PNS withdrawal profile (47-48%) (see Table 4.2). The overall model was not statistically significant between sociodemographic characteristics and ANS reactivity profiles at 36-months of age. The 36-month-old children who were Hispanic were 2.7 times the odds of having the reciprocal SNS activation/PNS withdrawal profile compared to the 36-month-old children who were not Hispanic (*OR* = 2.7(1), $p < 0.05$, *CI* = 1.00-7.25).

Discussion

In every selected sociodemographic characteristic category (biological sex, race, ethnicity, and FPL) at 18-months, the reciprocal PNS activation/SNS not activated profile was the most prevalent while at 36-months, every selected sociodemographic characteristic category had the reciprocal SNS activation/PNS withdrawal profile as most prevalent. At 18-months, children's race and FPL were significantly associated with all four ANS profiles and there were no significant relationships between children's sociodemographic characteristics and their ANS profiles at 36-months. Logistic regression models revealed significant relationships with the coactivation and coinhibition profile and FPL at 18-months and a borderline significant relationship between being Hispanic and the reciprocal SNS activation/PNS withdrawal profile at 36-months. These results partially supported study hypotheses.

This was a cohort study of racially- and ethnically-diverse, low-income children studied from the prenatal period through five years of age. The prevalence of children's racial background in the United States (U.S.) is 64% Caucasian, 16% Hispanic or Latino, 12% Black, and 5% Asian (Gatze-Kopp, 2018). In contrast to the U.S. population, our study sample of children living in the Bay Area of California, had a higher percent of Hispanic, African American, and Asian children. Furthermore, this sample included mostly children living in very low-income households, with the majority of participants < 200% of the FPL. Thus far, most ANS research studies include children from a middle- to high-income population that does not represent the national statistic that 21% of children under 6 years of age lives in poverty in the U.S. (National Center for Children in Poverty, 2019). As expected, race and ethnicity were significantly associated with each other; however, race nor ethnicity were associated with FPL. This predominately low-income sample allowed for the sociodemographic characteristic of ethnicity and race to be included in the analyses whereas in other studies they would not have been represented due to their correlation with income. By uniquely having an under-represented sample, this study leveraged that position with the attempt to understand relationships that may not otherwise have been captured.

The only significant relationship with regard to child's sex is its distinction for children with and without the coactivation profile at 18-months. There were almost half as many girls than boys with coactivation profiles which shows that boys were more likely to have a coactivation profile at 18-months of age than girls. This finding is congruent with some studies that reported that school-aged boys were more sympathetically activated compared to school-aged girls (Bush et al., 2011; Matthews et al., 2002). However, the majority of research has not found sex and ANS reactivity differences and that is consistent with the majority of this research study's findings.

Child's race was significantly associated with the coactivation profile at 18-months as there were more African Americans than Caucasians represented in this relationship. This finding would be congruent with previous research that found a relationship between African American children who were more likely to have greater PEP reactivity than White children (Wagner et al., 2016; Salomon, Matthews, & Allen, 2000). Being Hispanic had a borderline significant relationship with the classic reactivity profile at 36-months of age. This differs from the moderating effect of the presence of social support related to less reactivity to stress conditions in a primarily Latino sample (Alkon et al., 2011).

A child's FPL had the greatest number of associations with ANS profiles of all sociodemographic characteristics. Children who lived in families < 200% of the FPL were likely to have the coactivation and coinhibition profiles compared to children who lived in families > 200% of the FPL at 18-months. This finding supports El-Sheikh et al., 2009 study that found these two profiles associated with children's poverty level. In our study, children living in poverty were significantly more likely to have a negative PEP reactivity score at 18-months of age which highlights the sympathetic activation these children are experiencing. Others have reported this increase of PEP reactivity in young children (Alkon et al., 2011; Ellis, Essex, & Boyce, 2005). The concern is that children who have a developmental trajectory of increased SNS activation may eventually experience a dampened SNS response to stress which can put the child in danger if not able to appropriately respond to a stressful encounter (Evans & Schamberg, 2009; Evans & Kim, 2007).

Strengths and Limitations

Although this study has the strength of studying ANS reactivity in a diverse, low-income sample of young children and the inclusion of both SNS and PNS measures, there are some limitations to the study. The small sample size limited the statistical power to find significant associations within the logistic regression models. There is also a potential for measurement bias since the questionnaires were self-administered. This sample is over-represented with mothers who were overweight or obese during their pregnancy and some of these associations may be related to children's BMI and adiposity not included in these analyses.

Future Directions

These study results support the need to replicate the study design to identify if the study results can be used to inform the creation of an intervention. A possible intervention could be developed for childhood and adolescents who experience early childhood poverty and have a negative PEP reactivity score by three years of age. This early intervention could potentially reduce the stress associated with living in poverty and lower their sympathetic responses to everyday challenges. However, careful consideration should be made towards interventions that may lead children to be less adaptive to their environment. Future studies could explore more sociodemographic characteristics of the children for a more holistic understanding of the early childhood experience. Recruiting larger sample sizes will allow for greater statistical power to provide evidence of significant relationships. The authors acknowledge that there should be a high level of sensitivity when discussing the results of these findings with researchers and community members. Conducting ethical research is important for all researchers and the results need to be shared with space for feedback from the community who participated in the study.

Conclusion

ANS reactivity profiles and their relationship to sociodemographic characteristics can be a particularly useful phenomenon to understand the mechanisms that affect ANS regulation. These insights can create pathways for successful interventions that can alleviate the learning, behavioral, and health deficits that can result from ANS dysregulation. This study strives to move the field of ANS stress physiology forward by creating an awareness of the possible relationships between sociodemographic characteristics and ANS reactivity profiles. This evidence should be utilized with the intent of educating and addressing processes that are in need of change to promote the health and wellness of all people equitably.

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Table 4.1 Sociodemographic Characteristics by Autonomic Nervous System Profile at 18 Months

x^2(*df*), p-value	**Coactivation** n (%)	**Coinhibition** n (%)	**Recip PNS** n (%)	**Classic** n (%)	**Total** n (%)
Sex					
Boy	19 (16%)	10 (9%)	21 (18%)	6 (5%)	56 (48%)
Girl	10 (8%)	9 (8%)	33 (28%)	9 (8%)	61 (52%)
Total	29 (24%)	19 (17%)	54 (46%)	15 (13%)	117 (100%)
5.9(*3*), 0.12	4.8(*1*), 0.03*	0.2(*1*), 0.65	3.2(*1*), 0.07	0.4(*1*), 0.51	
Ethnicity					
Not Hispanic	14 (12%)	11 (9%)	30 (26%)	11 (9%)	66 (56%)
Hispanic	15 (13%)	8 (7%)	24 (21%)	4 (3%)	51 (44%)
Total	29 (25%)	19 (16%)	54 (47%)	15 (12%)	117 (100%)
2.56(*3*), 0.46	1.04(*1*), 0.31	0.02(*1*), 0.89	0.3(*1*), 0.86	2.0(*1*), 0.17	
Race					
Caucasian	7 (6%)	7 (6%)	17 (15%)	6 (5%)	37 (32%)
African American	10 (9%)	1 (1%)	5 (4%)	0 (0%)	16 (14%)
Other	12 (10%)	11 (9%)	32 (27%)	9 (8%)	64 (54%)
Total	29 (25%)	19 (16%)	54 (46%)	15 (13%)	117 (100%)
15.3(*6*), 0.02*	14.1(*2*), 0.01*	1.4(*2*), 0.49	1.8(*2*), 0.40	2.8(*2*), 0.24	
FPL					
<200%	13 (12%)	17 (15%)	36 (32%)	9 (8%)	75 (67%)
>200%	15 (14%)	1 (1%)	14 (13%)	6 (5%)	36 (33%)
Total	28 (26%)	18 (16%)	50 (45%)	15 (13%)	111 (100%)
12.5(*3*), 0.01*	7.6(*1*), 0.01*	7.1(*1*), 0.01*	0.8(*1*), 0.37	0.5(*1*), 0.5	

Recip PNS: reciprocal parasympathetic activation/sympathetic not activated; FPL: federal poverty level; *$p < .05$

Table 4.2 Sociodemographic Characteristics by Autonomic Nervous System Profile at 36 Months

$x^2(df)$, p-value	**Coactivation** n (%)	**Coinhibition** n (%)	**Recip PNS** n (%)	**Classic** n (%)	**Total** n (%)
Sex					
Boy	6 (6%)	17 (18%)	5 (5%)	17 (18%)	45 (47%)
Girl	4 (4%)	16 (17%)	3 (3%)	28 (29%)	51 (53%)
Total	10 (10%)	33 (35%)	8 (8%)	45 (47%)	96 (100%)
3.3(3), 0.35	FE $p = 0.51$	0.4(1), 0.51	0.9(1), 0.36	2.8(1), 0.09	
Ethnicity					
Not Hispanic	5 (5%)	21 (22%)	4 (4%)	34 (35%)	64 (66%)
Hispanic	5 (5%)	12 (13%)	4 (4%)	11 (12%)	32 (34%)
Total	10 (10%)	33 (35%)	8 (8%)	45 (47%)	96 (100%)
4.0(3), 0.26	1.4(1), 0.24	0.2(1), 0.65	1.1(1), 0.30	3.0(1), 0.08	
Race					
Caucasian	3 (3%)	13 (14%)	3 (3%)	16 (17%)	35 (37%)
African American	0 (0%)	5 (5%)	1 (1%)	5 (5%)	11 (11%)
Other	7 (7%)	15 (16%)	4 (4%)	24 (25%)	50 (52%)
Total	10 (10%)	33 (35%)	8 (8%)	45 (47%)	96 (100%)
2.7(6), 0.85	2.1(2), 0.35	1.1(2), 0.57	0.02(2), 0.99	0.5(2), 0.97	
FPL					
<200%	7 (8%)	18 (21%)	5 (6%)	35 (40%)	65 (75%)
>200%	2 (2%)	10 (12%)	3 (3%)	7 (8%)	22 (25%)
Total	9 (10%)	28 (33%)	8 (9%)	42 (48%)	87 (100%)
3.9(3), 0.27	0.05(1), 0.82	2.4(1), 0.12	FE $p = 0.4$	3.2(1), 0.07	

Recip PNS: reciprocal parasympathetic activation/sympathetic not activated; FPL: federal poverty level; FE: Fisher's Exact Test; *$p < .05$

Table 4.3 Logistic Regression Models of Autonomic Nervous System Reactivity and Profiles at 18- and 36-Months

18-months							
DV: PEP Reactivity							
Predictors	**B**	**SE**	**Wald**	**df**	**Sig**	**OR**	**95% CI**
Sex	0.30	0.41	0.54	1	0.46	1.35	0.60-3.03
Hispanic	0.02	0.41	0.001	1	0.97	1.02	0.45-2.27
FPL*	1.17	0.43	7.43	1	0.01	3.21	1.39-7.44
Constant	0.30	0.41	0.54	1	0.46	1.35	
DV: Coactivation Profile							
Predictors	**B**	**SE**	**Wald**	**df**	**Sig**	**OR**	**95% CI**
Sex	0.69	0.47	2.16	1	0.14	2.00	0.79-5.02
Hispanic	-0.51	0.46	1.24	1	0.27	0.60	0.24-1.48
FPL*	1.12	0.47	5.75	1	0.02	3.06	1.23-7.61
Constant	-1.62	0.46	12.25	1	0.00	0.20	
DV: Coinhibition Profile							
Predictors	**B**	**SE**	**Wald**	**df**	**Sig**	**OR**	**95% CI**
Sex	0.64	0.54	1.37	1	0.24	1.89	0.65-5.49
Hispanic	0.21	0.55	0.14	1	0.71	1.23	0.42-3.62
FPL*	-2.46	1.06	5.36	1	0.02	0.09	0.01-0.69
Constant	-1.65	0.53	9.58	1	0.002	0.19	
36-months							
DV: Classic Reactivity Profile							
Predictors	**B**	**SE**	**Wald**	**df**	**Sig**	**OR**	**95% CI**
Sex	-0.81	0.46	3.11	1	0.08	0.44	0.18-1.09
Hispanic*	0.99	0.50	3.87	1	0.049	2.70	1.00-7.25
FPL	-0.85	0.54	2.46	1	0.12	0.43	0.15-1.24
Constant	-0.16	0.47	0.12	1	0.73	0.85	

DV: dependent variable; PEP: preejection period; FPL: federal poverty level; **p* <.05

Chapter Five: Discussion

Summarization of Results

This study revealed novel information about the distribution, stability, and continuity of respiratory sinus arrhythmia (RSA) and preejection period (PEP) rest, challenge, and reactivity measures from 18- to 36-months of age in an under-represented, low-income, predominantly minority sample. RSA and PEP rest, challenge, and reactivity measures displayed normal distributions with little evidence of skewness or kurtosis at 18- and 36-months of age. In addition, there was moderate stability in the PNS and SNS resting and challenge responses within individuals over time but not in reactivity measures. There was a lack of continuity across the ages with significant mean increases of RSA and PEP rest, challenge, and reactivity from 18- to 36-months.

The distribution of young children in three of the four ANS reactivity profiles significantly differed between 18- and 36-months of age and the proportion of children with the reciprocal sympathetic activation profile was greater at 36-months than 18-months of age. There was a significantly different distribution of children among the ANS profiles from 18- to 36-months of age; although, there was some stability for one ANS profile, reciprocal PNS activation.

The relations between the sociodemographic characteristics (biological sex, race, ethnicity, and FPL) and ANS profiles showed that their prevalence differed at 18- and 36-months of age. At 18-months of age, the reciprocal PNS activation profile was the most prevalent profile for the four sociodemographic characteristics. On the other hand, at 36-months, the reciprocal SNS activation profile as most prevalent profile for the four sociodemographic characteristics. At 18-months, children’s race and FPL, not sex and ethnicity, were significantly associated with all four ANS profiles. At 36-months, there were no significant relationships between children's sociodemographic characteristics and their ANS profiles. Logistic regression models revealed significant relationships between < 200% FPL and the coactivation and coinhibition profiles at 18-months and a borderline significant relationship between being Hispanic and the reciprocal SNS activation profile at 36-months.

Discussion

The measures of RSA and PEP have been well-studied in childhood and their associations to childhood mental and physical health outcomes have been established (Boyce et al., 1995; El-Sheikh et al., 2009; Hagan et al., 2016; Treadwell et al., 2011). However, these studies did not include RSA and PEP at 18-month-olds which is a critical age in early childhood development. Repeated cardiac measurements of ANS reactivity in children younger than three years of age are rarely collected, leading to a gap in our knowledge of childhood developmental trajectories of ANS. In addition, the current body of research lacks diversity in their samples, specifically representation of non-White, non-Caucasian individuals, which creates another gap in our knowledge. Each year there are more studies that include ANS reactivity profiles, which provide an important way to summarize ANS findings across the two branches of the ANS. Future studies should continue to include these bi-directional profiles, according to Berntson et al., 1991, as these profiles can provide a way to translate the knowledge of childhood stress physiology to clinical practice. Lastly, sociodemographic characteristics such as biological sex, race, ethnicity, and socioeconomic status, may have a role in understanding the process by which the ANS responds and develops over time. To summarize, it is crucial to report on ANS measures' distribution, validity, and continuity to promote high-quality research that is relevant for dissemination, interventions, and clinical practice.

This dissertation study investigated these critical gaps in the literature by reporting on a) the distribution, stability, and continuity of RSA and PEP during resting, challenge, and reactivity states, in a sample of predominantly minority children from low-income families assessed longitudinally at 18- and 36-months of age, b) describing the proportion of children at 18 and 36 months of age in the four ANS reactivity profiles (i.e., 1) coactivation, 2) coinhibition, 3) reciprocal parasympathetic (PNS) activation, 4) reciprocal sympathetic (SNS) activation); and, assessing the stability of the four ANS reactivity profiles from 18 months to 36 months of age; and, c) identifying the relationship between selected sociodemographic characteristics (children's biological sex, race, ethnicity, and federal poverty level (FPL)) and children's ANS reactivity profiles (coactivation, coinhibition, reciprocal PNS activation, and reciprocal SNS activation) at 18- and 36-months of age.

The present findings were consistent with previous research in that RSA and PEP displayed a normal distribution, reflecting individual differences and a range of ANS activity across children at each age (Alkon et al., 2003; Alkon et al., 2011). There were approximately equal numbers of children with positive and negative RSA or PEP reactivity scores. On average, the children at 18-months may have begun the protocol stressed by the strange situation of having electrodes placed on their chest and starting an unfamiliar protocol. On the other hand, at 36-months of age the children appeared able to relax at the start of the protocol when an adult read a story aloud to them. In addition, the mean responses showed that many of the preschool-age children were able to return to their resting levels after the challenges were over and the adult read another story aloud to them.

In previous studies, RSA resting, PEP resting, and RSA challenge levels in infants and children have consistently shown stability over time (Alkon et al., 2011; Calkins & Keane, 2004) whereas, PEP challenge, RSA reactivity, and PEP reactivity have not shown stability (Hinnant et al., 2017). The results of this study align with Hinnant and colleagues' (2017) findings of moderate stability in 8- to 10-year-olds for RSA resting, PEP resting, and RSA challenge, but our study findings differed in our finding of moderate stability in 18- to 36- month-olds in PEP challenge measures. However, RSA reactivity was not stable between these age points, consistent with El-Sheikh and colleagues' (2017) 8- to 10-year-old findings and Alkon and colleagues' (2011) findings in a cohort of primarily Latino children from six months to five years of age. In this study, PEP reactivity also did not show stability between 18- and 36-months. This lack of stability in the PNS and SNS reactivity indicates the potential for plasticity in stress physiology during the early childhood period. Plasticity indicates individual changes in a child's stress responsivity from one time point to another (within-person variability) or changes over time in the stress response from person to person (between-person variability) (Hinnant et al., 2017). Our findings and others' support the theories of individual differences, such as biological sensitivity to context (BSC), in the stress response (Boyce & Ellis, 2005) as there are a range of ANS responses at each age group.

The statistically significant mean level changes of RSA and PEP resting, challenge, and reactivity from 18- to 36-months of age were indicative of developmental changes or a lack of continuity expected over time. Gatzke-Kopp & Ram (2018), Hinnant et al. (2017), Conradt et al. (2016), and Alkon et al. (2011) found more PNS withdrawal compared to rest for older children compared to younger children's longitudinal cohort samples. PEP resting and PEP challenge had larger mean change compared to the other measures which may suggest that the SNS response becomes more responsive over time. This finding suggests that older children may be more biologically responsive or sensitive to the challenges presented during the protocol or that they were more engaged in the ANS protocol compared to the 18-month-olds. Overall, the mean changes from 18- to 36-months of age are consistent with other studies of continuity from 6-months to 10-years of age (Alkon et al., 2006 & 2011; Bar-Haim, Marshall, & Fox, 2000; Bornstein & Suess, 2000a; Calkins & Keane, 2004). Other studies also support similar findings of increasing reactivity as the child ages (Alkon et al., 2006 & 2014).

There is consistency of the ANS reactivity profiles at 18- and 36-months of age. By 36-months, most children were classified in the classic reactivity profile and the lowest prevalence was for children classified as the reciprocal PNS activation, SNS not activated profile. There was a lot of movement in the prevalence of children characterized in each of the four profiles from one timepoint to the other. Our study also repeated a wide range of change from 5% to 58% of children having the same ANS reactivity profile at 18- and 36-months of age.

There was a larger percent of children at 36-months who had a negative RSA and PEP reactivity score than at 18-months. Studies have shown that as the child gets older (up until 5-8 years old), there is an increase in the number of children with RSA and PEP reactivity (Alkon et al., 2003, Alkon et al., 2011). This may be due to children becoming more engaged in the challenges in the protocol than children at earlier ages.

At 18-months, the majority of children were characterized with reciprocal PNS activation/SNS not activated profile, but at 36-months, the majority of the children were characterized with the classic reactivity profile and the reciprocal PNS activation/SNS not activated profile was the least frequent. The CHAMACOS cohort study found similar results from 12- to 42-months of age. The CHAMACOS study had 34% of their 12-month-olds exhibiting the reciprocal PNS activation, SNS not activated, but at 42 months, 40% of the children exhibited classic reactivity (Alkon et al., 2011). These findings were stable from 42- to

60-months of age (Alkon et al., 2011; Alkon et al., 2017). The continuity of the shifts in profiles does infer that there may be a developmental pattern to stress reactivity. Children with reciprocal PNS activation/sympathetic withdrawal (low ANS reactivity) were more likely to have sleep problems (Alkon et al., 2017) but protected against delinquent behavior in parental relationships with marital problems (El-Sheikh et al., 2009). This is an example of where having low reactivity for one child could be a positive attribute whereas for another child it could be viewed as a negative attribute. Externalizing disorders have been associated with children who have the classic reactivity profile (Pearson et al., 2005). However, one of the earliest studies of profiles has acknowledged that the classic reactivity profile is the most normative response to stress (Salomon, Matthews, & Allen, 2000).

Children at 18-months did not keep their same ANS profile at 36-months of age; thus, found a lack of statistical evidence for the stability of the profiles. However, the greatest number of children stayed in the classic reactivity profile from 18- to 36-months of age which supports the finding of those with a negative RSA or PEP reactivity score are more likely to stay with that score through 36-months of age. The reciprocal PNS activation profile did not have much representation at 36-months, but of those that did, 33% were that profile at 18-months. Our study did not come to any meaningful conclusions with the coactivation profile; however, the coinhibition profile had moderate stability and its representation almost doubled in number from 18- to 36-months of age. Not much is known about the coinhibition or coactivation profiles due to these profiles not represented in a larger percent of the population. Additionally, no significant changes or associations of coinhibition or coactivation have been found throughout the literature (Alkon et al., 2003). Coinhibition tends to be more prevalent in preschoolers but over time becomes less prevalent (Quas et al., 2014, Alkon et al., 2011). Coinhibition has shown to be a moderator in the relationship of experiencing marital conflict and delinquency in children and having the coactivation profile has shown to be a protective factor (El-Sheikh et al., 2009).

This was a cohort study of racially- and ethnically-diverse, low-income children studied from the prenatal period through five years of age. The prevalence of children's racial background in the United States (U.S.) is 64% Caucasian, 16% Hispanic or Latino, 12% Black, and 5% Asian (Gatze-Kopp, 2018). In contrast to the U.S. population, our study sample of children living in the Bay Area of California, had a higher percent of Hispanic, African American, and Asian children. Furthermore, this sample included mostly children living in very low-income households, with the majority of participants < 200% below FPL. Thus far, most ANS research studies include children from a middle- to high-income population that does not represent the national statistic that 21% of children under 6 years of age lives in poverty in the U.S. (National Center for Children in Poverty, 2019). As expected, race and ethnicity were significantly associated with each other; however, race nor ethnicity were associated with FPL. This predominately low-income sample allowed for the sociodemographic characteristic of ethnicity and race to be included in the analyses whereas in other studies they would not have been represented due to their correlation with income. By uniquely having an under-represented sample, this study leveraged that position with the attempt to understand relationships that may not otherwise have been captured.

The only significant relationship with regard to child's sex is its distinction for children with and without the coactivation profile at 18-months. There were nine more boys than girls with coactivation profiles which showed that boys were more likely to have a coactivation profile at 18-months of age than girls. This finding would be congruent with some studies that reported that school-aged boys were more sympathetically activated compared to school-aged girls (Bush et al., 2011; Matthews et al., 2002). However, the majority of research has not found sex and ANS reactivity differences and that is consistent with the majority of this research study's findings.

Child's race was significantly associated with the coactivation profile at 18-months. There were more African Americans than Caucasians in this relationship. This finding would be congruent with previous research that found a relationship between African American children who were more likely to have greater PEP reactivity than White children (Wagner et al., 2016; Salomon, Matthews, & Allen, 2000). Being Hispanic had a borderline significant relationship with the classic reactivity profile at 36-months of age. This differs from the moderating effect of the presence of social support related to less reactivity to stress conditions in a primarily Latino sample (Alkon et al., 2011).

A child's FPL had the most amount of significant relationships with ANS profiles than any other sociodemographic characteristic. Children who lived in families < 200% of the FPL were likely to have the coactivation and coinhibition profiles compared to children who lived in families > 200% of the FPL at 18-months. This finding supports El-Sheikh et al., 2009 study that found these two profiles associated with children's poverty level. In our study, children living in poverty were significantly more likely to have a negative PEP reactivity score at 18-months of age which highlights the sympathetic activation these children are experiencing. Others have reported this increase of PEP reactivity in young children (Alkon et al., 2011; Ellis, Essex, & Boyce, 2005). The concern is that children who have a developmental trajectory of increased SNS activation may eventually experience a dampened SNS response to stress which can put the child in danger if not able to appropriately respond to a stressful encounter (Evans & Schamberg, 2009; Evans & Kim, 2007).

Significance

This is the first known study to report on both RSA and PEP resting, challenge, and reactivity measures at 18-months of age. Developmentally, it is challenging to engage toddlers in specific tasks and to sustain their attention to measure resting states. Thus, this study contributes new knowledge at this age and compares their ANS responsivity at 18-months with 36-months of age.

This was the first study to evaluate ANS reactivity profiles at 18 months, and the longitudinal study design allowed the authors to characterize individual children's movement between profiles from 18- to 36-months of age. The significance of this contribution is heightened by the finding that this was a period of dramatic change in autonomic reactivity. These data are described in light of two complementary constructs, stability and continuity, that are important to report in a longitudinal developmental study and only requires at least two waves of longitudinal data. Another strength of the analysis is the findings compare with data from other studies collected at slightly younger and older age points, helping clarify the developmental trajectory of ANS profile distributions between six months and 7-8 years. This study also contributed data from a racially and ethnically diverse sample, whereas previous research has come from either a low-income Mexican American sample (Alkon et al., 2011) or more affluent, predominantly Caucasian samples (Alkon et al., 2006).

Limitations

Although this study contributed new findings to the field of ANS responsivity in young children, there are several limitations to consider. These findings are limited to two time points of repeated measures. While the SEED study does have RSA and PEP measurements from these same children at age six months, the 6-month ANS protocol utilized an attachment-based Still

Face Paradigm (SFP), rather than a developmentally challenging protocol like the 18- and 36-month protocols, and funding delays led to only half the cohort being assessed for ANS. Thus, the analyses conducted here were not appropriate to model starting during infancy. The challenges in the DCP were not randomized and the order of challenges may affect physiologic responsivity. Impedance cardiography is sensitive to movement and respiratory artifact (Bush et al., 2011; Zisner & Beauchaine, 2016). However, these ANS measures (RSA and PEP) offer an inexpensive, noninvasive estimation of central nervous system function as compared to EEG or fMRI studies.

In regard to the ANS reactivity profiles, it must be noted that the above versus below zero dichotomy used to classify the direction of children's PNS and SNS reactivity is imperfect and can lead to overly broad profile categorizations. The small sample size limited the statistical power to find large proportions of children in each sociodemographic group by each ANS profile and to find significant associations within the logistic regression models. There is also a potential for measurement bias since the questionnaires were self-administered. This sample is over-represented with mothers who were overweight or obese during their pregnancy and some of these associations may be related to children's BMI and adiposity not included in these analyses.

Future Research

To advance the field of ANS and standardize our findings, it is recommended that future studies use standardized, valid measures and methods for the assessment of ANS during resting and challenge conditions. For example, a protocol that assesses ANS responsivity including reactivity should be holistic and include a range of domains, such as the physical, emotional, cognitive, and social; and, these protocols should be repeated in the same children across time.

Administration of the 18-month-old protocol could be revised to illicit another measure of rest as many children may not have been relaxed at the start of the DCP since it was passive, and the child listened to an audio recording but didn't visually attend to a picture or book. Additionally, calculating reactivity scores separately by domain revealed that the greatest ANS reactivity was found with the physical domain. For example, the children had significant SNS activation and PNS withdrawal with the lemon taste challenge (physical domain). Future analyses of this domain-specific reactivity may be helpful for investigating how children at a certain age responds to a specific developmental contexts and possible environmental factors that may affect their physiologic response. This physical domain may also have specific utility for eliciting reactivity in situations where it is not possible to administer the entire four domain-specific DCP, or in a study designed with a specific outcome related to satiation or taste reactivity. Other researchers suggest that RSA and PEP measures be collected in conjunction with neuroimaging methods (such as fMRI, EEG, or PET) to assess neural correlates of autonomic functioning (Zisner & Beauchaine, 2016). Adding measures of genetic predisposition, environmental risk, and protective factors may help explain the interplay of endogenous and exogenous factors and their possible association with ANS reactivity.

Studies show that ANS profiles differ across samples and it is not known what factors contribute to these differences. Therefore, future studies should be designed to understand these differences in diverse samples. It could be due to a child's age, the type of protocol, or certain characteristics such as poverty or adversity; and, controlling for these factors in regression analyses could help support the hypothesis that these differences are due to developmental changes. Use of two separate categorization systems - Bernston et al. (1991) ANS reactivity profiles versus the ACM-guided patterns – made it difficult to interpret the results of these studies relative to one another. Assessing both categorization systems in parallel within several study samples could help clarify how the two systems relate to one another empirically (e.g., to quantify the degree of overlap between theoretically-similar “coinhibition” profile and “buffered” pattern). It would also be useful to evaluate whether one system’s categorizations are more strongly associated with outcomes of clinical interest (e.g. externalizing problems), so as to assess which system holds more promise to guide possible clinical interventions.

These study results support the need to replicate the study design to identify if the study results can be used to inform the creation of an intervention. A possible intervention could be developed for childhood and adolescents who experience early childhood poverty and have a negative PEP reactivity score by three years of age. This early intervention could potentially reduce the stress associated with living in poverty and lower their sympathetic responses to everyday challenges. However, careful consideration should be made towards interventions that may lead children to be less adaptive to their environment. Future studies could explore more sociodemographic characteristics of the children for a more holistic understanding of the early childhood experience. Recruiting larger sample sizes will allow for greater statistical power to provide evidence of significant relationships. There should be a high level of sensitivity when discussing the results of these findings with researchers and community members. Conducting ethical research is important for all researchers and the results need to be shared with the community who participated in the study.

Conclusion

A child’s neurobiological circuitry develops rapidly from birth to five years of age (Shonkoff et al., 2014). Understanding the measurable indicators that can affect the trajectory of these neurobiological pathways can reveal important information to affect change at earlier and more developmentally-sensitive time points. RSA and PEP are valuable measures to assess changes in ANS activity over time, with implications for understanding concepts such as biological embedding of early childhood experiences, biological self-regulation, and child adjustment, all of which can have important consequences for the health and wellness of an individual over their lifespan.

The analysis of ANS reactivity profiles between 18- and 36-months of age has helped fill in an age gap in the growing literature regarding developmental patterns of ANS reactivity. While previous literature suggested that a major shift in profile distributions occurred between 12 and 42 months (Alkon et al. 2011), this analysis narrows that age band and suggests that the shift occurs between 18 and 36 months. As the field of ANS research progresses toward clinical applications, this finding may inform optimal timing of interventions intended to lessen the effects of stressful experiences that occur during sensitive periods.

ANS reactivity profiles and their relationship to sociodemographic characteristics can be a particularly useful phenomenon to understand the mechanisms that affect ANS regulation. These insights can create pathways for successful interventions that can alleviate the learning, behavioral, and health deficits that can result from ANS dysregulation. This study strives to advance the field of ANS stress physiology by identifying possible relationships between sociodemographic characteristics and ANS reactivity profiles. These findings should be utilized with the intent of educating and addressing processes that are in need of change to promote the health and wellness of all people equitably.

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